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Genetic structure and migration of *Sitobion miscanthi* populations in China

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Genetic Structure and Migration *Sitobion miscanthi* populations in China

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Résumé

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Résumé : *Sitobion miscanthi* est un puceron nuisible aux céréales. Il s'agit d'une espèce dominante et migratoire en Chine pouvant se propager rapidement et occasionner des dommages majeurs aux cultures. La migration et la dispersion ne sont pas seulement des manifestations importantes de la dynamique des populations. Elles représentent des adaptations écologiques importantes face à des conditions environnementales variables. Le comportement migratoire est la principale raison de la difficulté de surveillance et de prévision de ces ravageurs. La température, le vent et la situation géographique sont les principaux facteurs abiotiques influençant la migration des pucerons. Dans cette thèse, les expériences au laboratoire et au champ ont été combinées sur base d'approches biologiques et génétiques. Premièrement, des paramètres du développement et de reproduction de différentes populations géographiques de *S. miscanthi* ont été déterminés en fonction de leur origine et de différentes températures environnementales. La durée de développement et la longévité des adultes de chaque population ont été réduites avec l'augmentation de la température. Aussi, aucune différence significative de capacité de reproduction n'a été observée au sein des populations nordiques. La latitude a été positivement corrélée à la longévité des pucerons. Des traits d'histoire de vie de ces populations au cours du développement ont été analysés. Ensuite, afin d'étudier l'impact de l'origine géographique de *S. miscanthi* en Chine et les facteurs affectant leur distribution, six populations géographiques ont été sélectionnées sur base des résultats biologiques afin d'analyser l'évolution de la différenciation génétique et de la dispersion parmi ces populations géographiques en utilisant des marqueurs moléculaires. Nous avons analysé 18 populations géographiques de Chine en utilisant un gène mitochondrial (COI), un gène nucléaire (*EF-1α*) et deux gènes endosymbiotiques de *Buchnera* (*gnd* et *trpA*). Les six populations de *S. miscanthi* ont été divisées en trois groupes, Yinchuan, Suzhou, et d'autres populations selon les trois gènes marqueurs génétiques.

Les résultats de l'analyse de la structure génétique basée sur le gène mitochondrial du puceron et les gènes de bactéries symbiotiques sont similaires. Ceci indique que des marqueurs symbiotiques pourraient servir dans l'étude de la structure génétique et de la diversité de la population des pucerons. Enfin, nous avons utilisé le génotypage par séquençage (GBS) pour identifier des polymorphismes de nucléotide unique (SNP) à l'échelle du génome afin d'en déduire la structure géographique et les schémas de migration. Deux principales voies de migration naturelles de *S. miscanthi* en Chine ont été déterminées : l'une va au sud du Yunnan au bassin du Sichuan, et l'autre des régions de Wuhan, Xinyang et de la péninsule de Jiaodong au nord-ouest.

En conclusion, *S. miscanthi* est un modèle de migrations saisonnières dans les régions productrices de blé en Chine. La poursuite de ces études permettra d'accroître la compréhension de l'adaptation et des migrations des pucerons de céréales, afin d'améliorer la surveillance et un contrôle efficace de ces ravageurs.

Mots-clés: *Sitobion miscanthi*, migration, population structure, temperature, *Buchnera*, molecular markers, SNP

Abstract

Jingxuan SUN (2021) ***Genetic structure and migration of Sitobion miscanthi populations in China*** (PhD thesis). Gembloux, Belgium, University of Liège, Gembloux Agro-Bio Tech, 138 p., 30 Tables, 14 Figures.

Abstract: *Sitobion miscanthi* (Takahashi), a wheat aphid, is an economically damaging aphid pest and the dominant species in China. Migration and diffusion are not only important manifestations of population dynamics, but also important ecological countermeasures for aphids to adapt to the environment. Migration, as the main physiological behavior characteristic of aphids, is the main reason for the difficulty in monitoring and forecasting. Temperature, wind and geographic location are the main abiotic factors for the migration of aphids. In this thesis, indoor and outdoor experiments were combined associated to methods of physiology and population genetics. Firstly, the physiological and reproductive parameters of different geographical populations of *S. miscanthi* at different temperatures and the life history traits of different populations during the growth and development period were investigated and analyzed. There was no significant difference in reproductive capacity within populations from northern China. In addition, latitude was positively correlated with aphids' longevity. Secondly, eighteen geographical populations from China were studied by using one mitochondrial gene *COI*, one nuclear gene (*EF-1α*) and two endosymbiotic *Buchnera* genes (*gnd* and *trpA*). Two main natural migration pathways of *S. miscanthi* in China were observed: one was from Yunnan to the Sichuan Basin and another from Wuhan, Xinyang and Jiaodong Peninsula areas to the northwest. We inferred that these aphids appear first in the southwest and south regions and spread to the north with the help of the southeast and southwest monsoons, which occur in spring and summer. In autumn, aphids spread southward with the northeast and northwest monsoons. Finally, to clarify geographical structure of *S. miscanthi* in China and the factors affecting its distribution, we selected aphid samples from six geographic locations in China and analyzed the evolution history of genetic differentiation. Our 6 geographical populations of *S. miscanthi* were divided into three groups, Yinchuan, Suzhou, and other populations. Genetic structure analysis based on the mitochondrial (*COI*) and the symbiotic genes (*gnd* and *trpA*) were similar indicating that the symbiotic genes could act as potential molecular markers in studying the genetic structure and diversity of aphid populations. Difference in annual average temperature may be the reason for diversity genetic structure of *S. miscanthi* providing theoretical evidence of the aphid migration. It was speculated that *S. miscanthi* has seasonal migration patterns in wheat regions to allow prediction of potential aphids outbreaks in China.

Keywords: *Sitobion miscanthi*, migration, population structure, temperature, *Buchnera*, molecular markers, SNP

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Jingxuan SUN

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List of Abbreviations

ABC	approximate bayesian computation
AFLP	Amplified fragment length polymorphism
AMOVA	analysis of the molecular variance
ANOVA	Analysis of variance
BYDV	Barley yellow dwarf virus
COI	Mitochondrial cytochrome oxidase I
COII	Cytochrome c oxidase subunit II
DNA	Deoxyribonucleic acid
GBS	genotyping by sequencing
GC	genetic clusters
HWE	Hardy–Weinberg equilibrium
ISSR	Inter-simple sequence repeat
MAT	mean annual temperature
ML	maximum likelihood
mtDNA	Mitochondrial DNA
ND5	NADH dehydrogenase 5
NDD	nymphal development duration
PCR	Polymerase chain reaction
QHL	Qinglin–Huaihe line
RAPD	Randomly amplified polymorphic DNA
rDNA	Recombinant DNA
RFLP	Restriction fragment length polymorphism
RH	relative humidity
RNA	Ribonucleic acid
SAMOVA	spatial analysis of molecular variance
SE	standard error
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
TDRI	Tropical Development Research Institute
trpA	tryptophan synthase, alpha subunit

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Chapter I: General Introduction

1.1. An overview of *Sitobion miscanthi*

1.1.1. Distribution range and biohazard of S. miscanthi

The wheat aphid, *Sitobion miscanthi* (Takahashi) (Hemiptera: Aphididae), is a major and widespread pest of cereal crops in China. *S. miscanthi* is almost exclusively anholocyclic with no sexual forms (oviparae and males) or egg stage. Reproduction is parthenogenetic and viviparous (Sekhar et al., 2001). *S. miscanthi* is Asiatic in origin and is widespread in China, India and the Far East and in the Western Pacific region (Gupta et al., 1994). There has always been confusion between *S. avenae* (Fabricius) and *S. miscanthi* (Takahashi) in China. Thanks to a systematic study of both aphid species, it was found that *S. avenae* is only distributed in the Yili region of Xinjiang, China, the aphids distributed in other parts of China that were originally named after *S. avenae* were *S. miscanthi* (G. X. Zhang, 1999, Qiao, 2009). *S. miscanthi* is a dominant species in most wheat zones with wide distribution and a wide range of hosts. In addition to damage to wheat crops, *S. miscanthi* also damages sorghum, rice, corn barley, oats and sugarcane (Wu, 2002). They have a board host range, high reproductive potential, strong genetic variation, and have diversified adaptation mechanisms to environmental conditions such as crops, climate change, and habitat. They often break out in large areas when environmental conditions are suitable. They can damage plants both directly by feeding on phloem sap, excreting honeydew. Moreover, aphids are also important insect vectors for spreading plant virus such as barley yellow dwarf virus (BYDV) (Gupta et al., 1994). BYDV can make disease-causing plants yellow, dwarf, and have fewer grains, which seriously affects the stable and high yield of grain in wheat areas, and causes great harm to agricultural production.

1.1.2. Life history of Sitobion miscanthi

Aphids are worldwide pests in various distribution areas. A lot of aphid species have shown remarkable adaptations by invading new habitats and crops (Mackay et al.,1993). Complete, incomplete and facultative cycles are the three common life history types. Complete cycle type aphids such as *S. miscanthi* not only maintain the characteristics of the species through the combination of male and female genetic material, but also produce differentiation within populations through genetic material exchanges between individuals (Owusu et al., 1996; Hales et al., 1997). Incomplete cycle type does not produce sex aphids in the life history, and there is no sexual reproduction. In the warm regions of central and southern China, *S. miscanthi* correspond to incomplete cycle type. Parthenogenetic reproduction occurs throughout the year as asexual aphid generations. Aphids overwinter on wheat seedlings or other grasses as parthenogenetic aphids. On the contrary, aphids belong to complete life cycle type in the cold northern regions of China (J. L. Chen, 2014). Reproduction is ensured by parthenogenesis with both apterous (wingless) and alate (winged) females

giving birth to live young. The apterae are adapted to exploit the host plants on which they develop, whereas alatae are adapted for dispersal over long distances and are responsible for the initial colonization of cereals and pasture grasses. Rapid reproduction can occur under favorable conditions, leading to population outbreaks. The alates are produced in response to overcrowding or when the plants have become nutritionally unsuitable (Watt, 2011).

S. miscanthi favors warmer conditions than other cereal aphids (Dixon, 1987). The curve of the population abundance of S. miscanthi from the winter wheat regions in China shows a bimodal shape with occurrence peak periods in spring and autumn. They begin to move and reproduce in spring when the temperature is above 6°C. When wheat is heading, aphids are transferred to the ears to damage, and the peak of aphids is reached in period of maturity grain filling of wheat above 16°C. When the temperature reaches 22°C, a large number of winged aphids appear. In parallel, the number of natural enemies increases, and the number of native aphids decreases, migrating to other places over the summer. After the emergence of wheat in autumn, the aphids return to the wheat field and harm the wheat. There was a second peak, but the damage was not serious as before. They overwinter in mid-November (P.H. Tang, 2013).

1.1.3. Study on the genetic diversity of Sitobion miscanthi

As in all living, insects not only maintain the relative stability of genetic traits and species, but also have some mutations during their long-term evolution. Genetic variation not only exists among insect species, but also among different populations of the same insect (Simon et al., 1996; Sunnucks, Driver, et al., 1997). There are some reports on the population diversity of *Sitobion*. Using 4 pairs of microsatellite primers to analyze the genetic diversity of *S. avenae* from several regions in Australia, it was proved that aphids did not have any sexual reproduction from a genetic point of view (Sunnucks et al., 1996). The genetic relationship between the periodic parthenogenesis and host specialization of *S. avenae* was revealed by using microsatellite loci to analyze the genetic structure of aphid populations (Sunnucks, De Barro, et al., 1997). The microsatellite detection (5 pairs of primers) of 8 geographical populations of *S. avenae* in France showed that the geographical distribution had a certain relationship with the choice of reproductive mode (Simon et al., 1999). Using 7 pairs of microsatellite primers, genetics studied that examine the genetic diversity of several indoor cotton aphid populations allow inferences to be made on obligate parthenogenesis was the main reproductive mode of cotton aphids (Fuller et al., 2010). Seven pairs of microsatellite primers were used to analyze the allelic diversity and heterozygosity of the sexual and clonal lines of *Rhopalosiphum padi*. The results show that these two parameters were quite different in various offspring (F. Delmotte et al., 2002). Using 4 pairs of microsatellite primers to study the genetic structure of 4 geographical populations of British grain aphid for 2 years, it revealed that the allele frequency was stable between geographical populations and time (F. Delmotte et al., 2002). Seven pairs of microsatellite markers were used to analyze the temporal and spatial genetic structure of different geographical

populations of *Myzus persicae* in France. Natural selection had an impact on the genetic structure of *M. persicae*, and the migration distance was judged to be 150km to 200km (Guillemaud et al., 2003). Twelve geographic populations of *S. avenae* had been tested for genetic polymorphism by 3 microsatellite loci (Guo et al., 2004) while five pairs of microsatellite primers were used to analyze the population genetic structure of four geographic populations of *S. avenae* in Beijing during 2 years to conclude that the growth strategies and migration rules were inferred (Y. M. Wang, 2007).

1.2. Basic theories and genetic parameters in population genetics

1.2.1. Population genetics

The number of organisms of the same species that live in a particular geographic area at the same time, with the capability of interbreeding is called a population. Individuals in a population are not mechanically grouped together, but are closely related to each other to produce a certain structure. Population structure is defined by the organization of genetic variation and is driven by the combined effects of evolutionary processes that include recombination, mutation, genetic drift, demographic history, and natural selection. Population genetics is the study based on the principles of genetics, using mathematical and statistical principles and methods to study the non-random distribution pattern of the gene or genotype frequency of the population in time and space and its natural selection and mutation, recombination, genetic drift and gene flow (Hartl et al., 1999). The exploration of population genetic structure and its influencing factors is the basis for discovering biological environment adaptation, speciation process and species evolution mechanism.

1.2.2. Population genetic equilibrium-Hardy Weinberg's law

Hardy-Weinberg law is an equation that describes the genetic balance within a population. It was discovered independently in 1908 by Wilhelm Weinberg, a German physician, and Godfrey Harold Hardy, a British mathematician. Both were pioneers in mathematically illustrating this principle also referred to as Hardy–Weinberg equilibrium, theorem, law or model. The law means that under random conditions, the population is large enough, and the individuals of the population mate randomly, there is no mutation, no selection, no migration, no genetic drift, and the frequency of each allele keeps the gene constant from one to another generation (Hartl et al., 1999). This law is widely used in biology, ecology, and genetics. Since then, the mathematical foundation of population genetics and related algorithms have gradually formed, and the theoretical system of population genetics has been initially established.

1.2.3. Features of molecular evolution

Molecular evolution is the evolution of biological macromolecules in the process of biological evolution. It mainly focuses on the relationship between changes in the structure of biological macromolecules and biological evolution during the development process of biological evolution (Gillespie, 1993). It mainly includes the evolution of protein molecules, nucleic acid molecules and genetic code. The study of molecular evolution can provide evidence for the biological evolution process and in-depth study of evolutionary mechanisms.

1.2.4. Molecular clock and neutral theory

The discovery of molecular clocks and the proposal of neutral theory have greatly promoted research in evolution, especially molecular evolution. This filled the gaps in the understanding of molecular evolution. After that, a series of theoretical methods that rely on nucleic acids and protein sequence information were established (Takezaki et al., 1995). Molecular evolution research helps to further clarify the molecular basis of species evolution, explore the mechanism of gene evolution, and study the relationship between gene sequence and function from the view point of gene evolution.

Studies had confirmed that the evolution rate of specific proteins or DNA fragments in each subfamily remains relatively constant, that was, the rate of evolution at the molecular level without relation with the length of biological generations and the environmental conditions for survival and population size. Based on the constancy of molecular evolution rate, molecular clocks of species could be established based on known molecular phylogenetic and paleontological data (Francisco et al., 1999; Bromham et al., 2003; Kumar et al., 2005).

Most mutations are neutral and do not distinguish between favorable and unfavorable theory. Then, these mutations will not occur in the situation of natural selection and survival of the fittest. Biological evolution is mainly the result of neutral mutations in natural populations of random genetic drift, but no related to natural selection (Kimura, 1983). Under neutral theory, a majority of mutations that arise spontaneously are neutral, and it (loss or fixation) is primarily determined by drift. Only after further morphological and physiological differences could natural selection play a role (Ohta et al., 1996; Wagner, 2008).

Genetic drift is the phenomenon that the gene frequency in the population changes due to the random combination of male and female gametes during reproduction, which is associated with bottleneck. This is the basis of the neutral theory. The smaller the population is, the greater the influence of genetic drift on the allele frequency of the population is. Genetic drift keeps some genes and loses some genes. This phenomenon has unrelated to the organisms, and it is almost entirely dependent on random effects (Masatoshi et al., 2005; Alonso et al., 2006; Leigh, 2010).

1.3. Migration of aphids

Many aphids have long-distance migration behavior (Taylor, 1977, 1986; Hugh D. Loxdale et al., 1993). The migration pattern, flight behavior, relationship between flight and reproductive development, effects of nutrition and temperature on flight of cereal aphids have been investigated in considerable detail in America and Europe (Dixon et al., 1993). *Schizaphis graminum* could be carried by the monsoon from the south of the United States to the north and even as far as Canada (Kring et al., 1988). *Macrosiphum avenae* could move with the southwesterly airflow from the winter wheat area in the northwest to the spring wheat area in Inner Mongolia and other places in May, and the incidence of wheat virus disease in the immigration area was closely related to the emigration area in China (G. X. Zhang et al., 1985). Wheat aphids generally move northward with the southwest wind from March to June, and then move south with the northwest wind after August, and then become the source of insects on autumn seedlings in winter wheat areas. *M. avenae* had been found to migrate seasonally in the mountainous areas of northern and southern China. The following is an introduction to the migration behavior of aphids, the ecological factors that affect the migration, and research methods of aphids' migration.

1.3.1. The migration behavior of aphids

Aphids, a long-distance migratory pest, can rely on their own flying ability but also use air currents to quickly disperse and harm crops. Migration gives aphids the opportunity to choose more suitable host plants and physiological conditions, so that the scope of crop damage gradually expands. The "S" curve is a commonly used takeoff trajectory for aphids, and the desire to take off is affected by its own developmental status, host plants and climatic conditions. *Rhopalosiphum padi* in China and Northern Europe migrate in winter and summer. The leaves and extracts of *Prunus padus* that have been damaged by aphids could disperse the takeoff and migration of aphids, while healthy leaves and extracts cannot stimulate takeoff. The substance that stimulates takeoff is methyl salicylic acid (Ninkovic et al., 2021). The load of the wings affects the take-off of the aphids, but this capacity of different aphids is different. *Cinara pinea* could take-off to twice the weight of the wings that can restrain other aphids from taking off (Kidd, 1991). The short-distance spreading behavior of aphids mostly occurs at low altitudes, and the long-distance migration can even reach the atmospheric boundary layer. Peach aphid, *Aphis nasturtii*, *A. frangulae*, *Brevicoryne brassicae* and pea aphid, plum large aphid *Hyalopterus pruni*, long wheat aphid, wheat straw aphid, *R. insertum* was detected with the electric worms and yellow plates at an altitude of 12.2 m in Aschersleben, Germany. This evidence suggested that these aphids could fly at least 12.2 m above the ground (Schmidt et al., 1988). Cotton aphids were caught with a sampler supported by a kite balloon at an altitude of 150m in different seasons in West Bengal, India (Reynolds et al., 1999). *Phorodon humuli* could migrate to a distance of 1 km, and some individuals could even ascend to the top of the warm layer and travel farther under suitable conditions.

Five-year observations have shown that about 20 species of aphids migrate across parts of the southern Baltic Sea each year, and many aphids were trapped in the traps on the lighthouse 50 meters from the Swedish seashore (Wiktelius, 1984). In the 1990s, it was discovered that aphids were distributed in layers on the inversion layer of the atmosphere through aerial capture, indicating that the aphids migrated to the central and western regions of Illinois and moved in the atmospheric planetary boundary layer (Isard et al., 1990). There are few direct studies on the process of aphids landing on host plants. The short-distance low-altitude migration and landing may be guided by the physical and chemical signals of the aphid itself to the host plant, but the randomness was high. The colonization behavior of *R. padi* to the *Prunus padus* tree in autumn was attracted by the aggregation pheromone which was produced by female aphids (Pettersson, 1993). But in southern Scotland, only 0.6% of *R. padi* migrating in autumn could find a host plant (Ward et al., 1998). In Washington, *P. humuli*, a major pest in summer on the hop tree, overwinters on plum trees in winter. In the hop tree planting area, more aphids were attracted in the fall than in the spring, but in the non-planting area the spring migration period could attract more aphids (Wright et al., 1995). After migrating to a new host site, this aphid tended to settle and reproduce without migrating further long distances. In addition, the odor of leaves could produce selective behavioral responses to winged aphids in a small area (Pettersson et al., 1998). This could be used as a signal for the local adjustment of the aphids to find the host after landing in a large area.

1.3.2. Ecological factors affecting the migration of aphids

Environmental factors such as temperature, humidity, light, wind and rainfall can affect the migration behavior of aphids. Due to the small size, light weight, and limited flying ability of aphids, temperature and wind become the two most important factors affecting the migration.

1.3.2.1. Effect of temperature on migration

Temperature is one of the important life activity elements that affect the growth and development, feeding, reproduction and migration of insects. Migratory insects take long-distance migration as an adaptation to the external environment, so that they can avoid bad environments and choose new habitats for colonization. Temperature is one of the inducing factors of migratory behavior. Migratory insects usually choose the right temperature and take off on a large scale together (Walker, 1980). The suitable temperature and humidity for flight of *S. avenae* are 12-22 ℃ and RH 60%-80% respectively. Within this range, the flight speed will increase with the increase of temperature or humidity; if the temperature is lower than 8 ℃ or higher than 25 ℃, its flight ability is significantly reduced (Cheng et al., 1997). There were no individual takeoffs of *S. avenae* within 16 h at 10 ℃; 70% of individuals took off at 15 ℃; when the temperature reached 20 ℃, 100% of them took off. Strong wind would delay takeoff, but it couldn't be forbidden (Dean, 1974). Principal component analysis was used to infer that the main factors affecting the migration of *M. persicae* were relative humidity and rainfall, and the

peak period of migration was from late April to early May. When the temperature in western Hungary reached 30℃, the aphids no longer migrated. The low temperature and 59.7 mm rainfall in May would inhibit the migration of the aphids on the leaves (Kuroli et al., 1988). Studies on the seasonal changes in the aphids population abundance of potato fields in Hokkaido, Japan show that cotton aphids could occur on potatoes, and the time of migrating into potatoes was in mid to late June, and they would migrate frequently (Nakata, 1995). The migration time of beet aphid from winter host to soybean field occurred in early May in Alsace (Zhu et al., 2006). *Cavariella aegopodii* migrated from the winter host willow to the radish and parsley in May, and then migrated back to the willow at the end of July. This is closely related to the temperature and air humidity at this time (Ghosh et al., 1986).

1.3.2.2. Effect of wind on migration

Wind speed and wind direction have a great influence on the migration path and distance of aphids. From the results of the hanging test, it could be found that the flying ability of aphids is quite limited, but it can still migrate long distances, which is the effect of wind. There have been many observations about the effect of wind in the migration process of aphids. *S. avenae*, *Rhopalosiphum maidis* and *R. padi* usually cannot overwinter in South Dakota of United States. The insect sources migrate from the south, and the aphids appear from April to May with low-level southerly jets in the south (Kieckhefer et al., 1974). The spreading behavior of cotton aphids in the field is mainly affected by the terrain, because the terrain will affect the wind direction and wind speed, resulting in uneven distribution of cotton aphids in the field. The essence is that the wind affects the spread of cotton aphids (Zhu. et al., 1956). Wind force and wind direction affect the migration and migration direction of *Therioaphis trifolii*. On days with unidirectional wind, on the sticky board on the direction of the wind and the flight path of the aphids, 95% of the total aphids are stuck to the aphids, while on the wind direction changing days, aphids could be caught on all sticky insect boards, and there was no difference in the number. The fact is that wind affects the spreading behavior of cotton aphids (Tamaki et al., 1972). Many aphids, such as the peach aphid, migrated long distances from other countries to Sweden by air transport (Wiktelius., 1977). Using the differences in the response of pea aphids to photoperiods in different latitudes and weather analysis, it was found that Manitoba's aphids migrated from the south 300 km, and the migrated populations were likely to be carried by the southern airflow 24 to 36 hours ago (Smith et al., 1989). With the wind, the migration of aphids is no longer hindered by the Pacific Ocean and high mountain peaks. If there is a 1m-long front in a wheat field, it could transport 133 million aphids every minute to 2m. The migration time of aphids is concentrated in the time when the light is brighter, and in warm and windless weather, the aphids spread around the ground surface. When the wind speed reaches 6 km/h, they can fly to an altitude of 10m or higher, and strong winds can bring the aphids population to an altitude of 1km. *Schizaphis graminum* also moved from the south of the United States to the north and even Canada with the monsoon (Tofangsazi et al., 2010). The results of multiple experiments have

shown that wind plays an important role in the migration of aphids.

1.3.3. Research methods on aphids' migration

The methods of observation and study of aphids migration have been reported in many places in the world and includes insect population observation, aphids physiology, ecology and molecular biology (G. X. Zhang et al., 1983). The observation of the number of aerial insects requires tools such as traps or radars. There are three types of traps most used: one is filter traps, such as John traps or Taylor traps. The trapping rate of this type of trap is poor at low wind speeds, and it is required to be used in weather with wind speeds higher than 10km/h; the second one is sticky board trap, such as Malaise board or Shands board; the last one is the yellow trap, which is usually placed on top of the crop, suitable for trapping *M. persicae* and the wheat aphid *S. avenae*. The newer trap was invented by Johoson, Taylor's improved high-altitude fluke, suitable for collecting aphids at an altitude of 12m (Mcconn et al., 1997).

Measuring the differences in the response of aphids to photoperiods in different areas can also be used to determine the source of aphids. An example of this is the study carried out it is found that the pea aphid migrated 300km from the south to Manitoba, Canada (Smith et al., 1989). There is also the use of ovarian dissection and hanging flying methods to determine whether the winged aphids are migratory and their migratory ability is strong or weak. The takeoff angle measurement is used to determine the potential energy of aphids taking off. Generally, the larger the takeoff angle, the greater the potential for migration (X. D. Liu et al., 2004).

During years, entomologists and ecologists have used radar for monitoring and early warning of biological disasters, especially migratory pests (Cheng et al., 2005). Since it was discovered in the early 1950s that radar could detect microwave signals reflected by aerial insects, insect radar has been highly valued by experts. Bent from the Rothamsted Experimental Station in the UK has developed a beam radar to automatically monitor the migration of aphids (Bent, 1984). At the same time, Tropical Development Research Institute (TDRI) produced prototypes for monitoring medium and large insects, and conducted observational experiments (Reynolds et al., 1997). In Finland, a study using Doppler weather radar allowed to observe many aphid migration. However, it was difficult to count the number of aphids by the radar echo signal. Individual aphids were too small to be easily monitored and they migrated together (Lu et al., 2004). Also, the calculation and analysis of radar data is very cumbersome which cannot be mastered by ordinary technicians, limiting the development of scanning insect radars for long-term monitoring (Cheng et al., 2005). In addition, millimeter wave radar is expensive (generally working wavelength is 8mm), and it is not cost-effective to arrange enough aphids on the possible migration path. In recent years, the rapid development of molecular biology technology and the combination of entomology have provided new means and methods for the study of aphids' migration. The analysis of the geographical distribution of genetic variation of aphids can infer the haplotype distribution and reproductive isolation of aphids in

different regions, and then analyze the possible migration paths, which could provide molecules genetic evidence for studying the migration and long-distance virus transmission. Jongsma *et al*. (1995) inferred that the corn aphids in Illinois did not migrate from southern Louisiana, Texas and Oklahoma using molecular markers (Jongsma et al., 1995). Delmotte *et al*. (2002) analyzed the population genetic structure of *R. padi* using isoenzyme electrophoresis and microsatellite markers (François Delmotte et al., 2002). Yan and Simon (2001) studied the polymorphism of 8 microsatellite loci in 55 parthenogenetic strains of *R. padi* in France (Yan et al., 2003) while Liu *et al*. (2001) analyzed the genetic polymorphism of *Diuraphis noxia* in different geographical populations and analyzed the population genetic structure using microsatellite markers. Also, Dedryver *et al*. (2001) analyzed the genetic variation of the two populations in different years. According to the different composition of genetic variation, it was speculated that there was the possibility of the migration of exotic aphid sources. Li *et al*. (1998) used 21 random primers to apply to *S. avenae* in 5 regions using RAPD markers. After cluster analysis, they found that the genetic difference between the Shijiazhuang population and the Yantai population with large geographic differences was very small. It was speculated that it may be affected by the monsoon that caused the long-distance migration of *S. avenae*. Llewellyn *et al*. (2003) used 4 microsatellite polymorphism sites to analyze the population structure of the *S. avenae* and found that there were huge population genetic differences between the same sampling site and different years. Combined with meteorological data, it was pointed out that the immigration and emigration of aphids may be the most important factor in population genetic differences (Llewellyn et al., 2010).

The long-distance migration of aphids gives them the opportunity to choose a more suitable host plant and living environment. Therefore, the migratory behavior and rules of aphids are particularly important in preventing and controlling the spread of plant diseases and further damages. Due to the small size of aphids, traditional marking and manual recycling methods are not suitable. Net catching, overwintering surveys and insect radar to monitor the migration of aphids also had several limitations. Therefore, at present we believe that the most feasible method is to use molecular genetic marker technology to analyze the genetic structure of aphids in different geographic populations, to study the gene communication among the populations, to detect their gene flow and to infer the migration path and source of the aphids may be more convincing.

1.4. Research of molecular genetic marker techniques in genetic diversity of insects

1.4.1. Introduction on molecular genetic markers

Genetic diversity is an important part of biodiversity (D. M. Ward et al., 1998;

Costa, 2003; Meirmans et al., 2004; Prentis et al., 2010). The genetic material in the cell is the carrier of biological genetic information whose mutation leads to the generation of genetic diversity, enabling the species ability of evolutionary adaptation (Klein et al., 1988; Whitehead et al., 2006; Cerón-Carrasco et al., 2015). There are three kinds of genetic material variation: chromosomal variation, mutation and recombination.

Methods for detecting genetic diversity have been continually refined, from morphological, cytological, physiological and biochemical to molecular levels (Reski et al., 2015). The purpose is to reveal the variation of genetic material between different organisms or different populations. Currently, every method of detecting genetic diversity has its own advantages and limitations both in theory and in practical researches. Morphological markers are a way to distinguish individuals by using the differences of external morphological characteristics. The variation of chromosome number includes ploidy and aneuploidy. DNA molecular marker is a genetic marker method based on the variation of DNA molecular base sequence, which directly reveals the polymorphism at the level of genomic DNA. Therefore, it is difficult to find one to replace others and multi methods and technologies, could provide valuable information and help people understand genetic diversity and its biological significance.

Insects molecular phylogenetics, the study of evolutionary relationships among organisms using molecular sequence data, has grown rapidly based on population genetic structure, phylogenetic evolution and species identification. With the advance of polymerase chain reaction (PCR) and bioinformatics, insects molecular phylogenetics has grown rapidly based on population genetic structure, phylogenetic evolution and species identification. At present, more studies have focused on molecular markers worldwide.

1.4.2. Molecular markers used in insect research

Genetic markers can distinguish different individuals or populations by identifying the inherited material or traits (Bowers et al., 1997; Hauskeller et al., 2004). The earliest genetic markers were morphological followed by cytological ones with the support of microscope technology. In the 1960s, isozyme label was developed based on electrophoresis technology (Wick et al., 1990). These three markers are gene expression type markers, with fewer polymorphic sites available, which are easily affected by environmental conditions and developmental stages of insect. In the 1980s, molecular makers appeared detecting the differences in DNA sequences with the advantages of high stability, less influence by environmental conditions, high information content and wide comparability among different population and groups. Therefore, it has become another powerful tool in genetic markers and has a unique advantage in revealing the genetic diversity within or between species (Z. Liu et al., 1993).

1.4.2.1. Restriction fragment length polymorphism (RFLP)

RFLP was proposed by Bostein *et al*. (1980) to construct plant genetic maps and refers to the variations in the length of genotypic restriction fragments caused by restriction site mutations, increase, decrease, rearrangement, insertion and/or deletion (Montes et al., 2008). In RFLP, DNA is digested by restriction enzymes, producing small fragments, which reflect the distribution of restriction sites. RFLP are stable in different insect development stages, phenotype and environments. However, RFLP also has shortcomings: the operation is complicated and time-consuming; RFLP has only one or two polymorphic sites, reflecting/showing low polymorphic information (Williams, 1990); RFLP is suitable for single or low copy genes with species specificity (Sang et al., 2002); the application of radioisotopes is harmful to humans.

1.4.2.2. Amplified fragment length polymorphism (AFLP)

AFLP can detect more polymorphic fragments in one selective amplification, which can reveal the DNA polymorphism to the greatest extent. So it plays a great role in insect classification and evolution research (Vos et al., 1995). However, AFLP technology is not suitable for classification studies at the genus and family level, because there are only a few common bands between genera or species at a similarity level of less than 40%, and unrelated species will be clustered together. Then, AFLP analysis technology is more suitable for closely related species, such as the structure and differentiation of populations (D. Campbell et al., 2010). AFLP had been used to identify *Spodoptera frugiperda* collected from rice and corn. Individuals collected from rice showed greater variation than those collected from corn (Pashley et al., 2004).

1.4.2.3. Randomly amplified polymorphic DNA (RAPD)

RAPD is a DNA molecular marker technology proposed by William et al. and Welsh & McClelland at the same time (Lynch et al., 1994). RAPD mainly use random primers of 10 artificially synthesized oligonucleotides to amplify the genomic DNA (Welsh et al., 1995). When base mutations, insertions or deletions occurred in target fragment, the amplified products changed in the size and quantity, reflecting the DNA polymorphisms in the corresponding regions of the genome. The experiment operation of RAPD is simple and fast. The amount of genetic information is large, the polymorphism is high; RAPD random primers are versatile and extensive. RAPD molecular markers have been widely used in the study of insect genetic diversity.

1.4.2.4. Simple sequence repeat (SSR)

SSR, also known as Minisatellite DNA (Minisatellite) was proposed and established by Wahls et al. (1991). Generally, there are repetitive DNA sequence consisting of 1 to 6 nucleotides with two conserved and specific ends. Specific primers can be designed according to the sequences at the two ends to amplify

and obtain the length polymorphism. Compared with other molecular markers, SSR is co-dominant, consistent with Mendelian inheritance and high polymorphism. SSR is favored by many researchers and require sequence analysis and cloning of microsatellite sites, which is time and money consuming (Mccouch et al., 1997).

1.4.2.5. Inter-simple Sequence Repeat (ISSR)

ISSR is established by Zietkiewicz (Ewa et al., 1994). One or four bases were added at the 3' or 5' end of the simple repeat sequence as primers to amplify a fragment of a reverse sequence of SSR, and evaluate the polymorphism of the sample according to the specificity of the size of the amplified product fragment (X. Wang et al., 2007). ISSR is compatible with the advantages of many molecular marker technologies: it does not require pre-designed primers like SSR and it has high safety, simple experimental operations. The quality requirements for DNA are not high and the cost is low. ISSR are more stable, faster and more reproducible compared with RAPD (Piao et al., 2004). It is widely used in the study of insect genetic diversity (Nkongolo et al., 2005).

1.4.2.6. Single nucleotide polymorphism (SNP)

SNP mainly refers to DNA sequence polymorphisms at the genome level caused by variation of a single nucleotide, including single base deletion, insertion, conversion and transversion (Baird et al., 2008). SNP is of high genetic stability, abundant sites and wide distribution, easy typing, fast detection, and easy automated analysis. SNP is highly valued by systematic evolution researchers and molecular breeders for its advantages, and has become an indispensable tool in life science research (Ganal et al., 2009).

1.4.3. Molecular markers genes for phylogenetic analysis among populations

Morphological characteristics are the most important basis for insect classification. However, some of these are unstable or have multiple variability in some insects, which brings great difficulties to identify species. In recent years, the molecular markers have gradually been used to distinguish closely related species, identify difficult species and construct phylogenetic trees. The molecular marker genes used for phylogeny analysis are mainly mitochondria gene, ribosomes gene, symbionts gene and SNP (Gueguen et al., 2010; De Mandal et al., 2014).

1.4.3.1. Mitochondrial genes

Mitochondrial DNA (mtDNA) represents only a tiny fraction of organismal genome size, yet it has been by far the most popular marker of molecular diversity

in animals over the last three decades (Roger et al., 2017). It is widely used in studies of insect phylogeny and population structure variation. It is also used to identify relative species and sub-species taxa that are difficult to distinguish external morphological features. Chu et al. (2005) sequenced a large number of *Bemisia tabaci* (Gennadius) ribosomal and mitochondrial genes, analyzed the genetic differentiation of different geographical populations of *B. tabaci* and divided into different geographical groups. Du et al. (2008) found that the genetic relationship between *Limaria sativa* from different host populations is related with preference for the host based on the ribosomal and mitochondrial genes sequencing.

1.4.3.2. Ribosomal genes

Ribosomes are compact ribonucleoprotein particles composed of dozens of proteins to synthesize proteins (Gautier et al., 1998). The non-transcribed spacer is easy to evolve, while the transcribed spacer is slightly conserved. The variations in spacer sequences are easily fixed under low selection pressure and the tendency of rapid reorganization, showing high differences. The internal transcribed spacer does not participate in the formation of ribosomes, it is subject to low selection pressure and rapid evolution, so it can be used for the study of species, subspecies, and even individuals, as well as hybrid phenomena and population genetic structure (Zheng et al., 2007). Ribosomal gene sequence can be used to distinguish species with similar morphologies. Using the ribosomal gene analysis of *B. tabaci* in South Korea, China, Ben, Israel, France and other regions were divided into two biotypes. The ribosomal ITS proved to be a more robust marker for aphid identification than suggested by previous studies. The genes were used to rapidly distinguish *Rhopalosiphum* species (Bulman et al., 2005). The most important limitation in studying insect phylogeny is that there are many variations in a single individual, and it is difficult or impossible to find homology. Therefore, the research population must review each species (Jorgensen et al., 1988).

1.4.3.3. Symbiotic genes

Insect symbiotic bacteria are divided into primary and secondary endosymbionts (T. Tsuchida, Koga, Shibao, et al., 2010). They provide essential nutrients for the host for first kind and may have an impact on the fitness, competitiveness, and evolution of the host for second type respectively. The gene sequences of primary symbiotic bacteria are enriched during the long-term co-evolution leading to important feature interactions (Shigenobu et al., 2000). The symbiotic relationship between secondary symbiotic bacteria and host insects is temporary, reflecting that the latter might infect the host insect at multiple times with horizontal transmission mode (Loxdale et al., 1998). Endosymbiotic bacteria may evolve from a free organism into an organoid, whose structure and function are similar to those of mitochondria and chloroplasts, and all have a layer of membrane wrapped from the host. It exists in the host's entire life cycle, replicates separately outside the host, has a different protein synthesis system from the host,

and is not resisted by the host's defense mechanism (Feldhaar, 2011; Vorburger et al., 2018). Many endosymbionts are transmitted vertically from the host's eggs or embryos to their offspring, affecting the evolutionary branch and population structure of endosymbionts. The endosymbiotic bacteria and the host will become an interdependent whole and evolve together. The genetic material of endosymbionts is different from that of free-living bacteria, which determines that endosymbionts are dependent on host cells (Wernegreen et al., 2002). Endosymbiotic bacteria cannot be artificially cultured outside the host at present (Degnan et al., 2009).

1.4.3.4. Single nucleotide polymorphisms

Single nucleotide polymorphisms, frequently called SNPs, are the most common type of genetic variation. The study of SNP has attracted extensive attention. The construction of genetic map with SNP loci as important information is of great significance to the study of insect biodiversity SNP is the third-generation genetic marker after AFLP and SSR markers. It has high frequency and stable inheritance SNP molecular markers have broad application prospects in entomology. Wondji et al. (2007) analyzed 50 gene fragments of 21 stream *Anopheles funestus* by SNP, and finally detected a total of 494 SNPs, of which 303 were in the gene coding region (including 5 insertion/deletion sites), they had high polymorphism. Morales-Hojas et al. (2020) studied cereal aphids from China and UK by using SNP.

1.4.4. Advances in molecular marker techniques and their applications in insects

Over the last 30 years, molecular marker techniques have been efficiently employed in entomological studies, such as genetic relationship among populations or individual, migration route, mating and reproduction behavior (Table 1-1).

Table 1-1. Applications of molecular markers in insect research

Biological problem	Molecular marker	References
Genetic relationship and phylogeny among populations	SSR	Estoup *et al*., 1995 England *et al*.,1996 Fuller *et al*., 1999 Llewellyn *et al*., 2003 Hou *et al*., 2006 Margaritopoulos *et al*., 2007 Liu *et al*., 2010
	mtDNA	Tsutsui *et al*., 2001

		Luo *et al*., 2002 Tuda *et al*., 2004 Albre *et al*., 2008 Shefran and Payton 2009 Xu *et al*., 2011
	AFLP	Niu *et al*., 2006 Timm *et al*., 2006
	RFLP	Mukha *et al*., 2007
	SNP	Wondji *et al*., 2007 Van *et al*., 2012
	RAPD	Angela *et al*., 2008
	Symbiont	Clark et al., 1999
Defining the relationship among species	SSR	Field *et al*., 1999 Zhang *et al*., 2009 Sun *et al*., 2012
	mtDNA	Webster *et al*., 2004
	RFLP	Qin *et al*., 2008
	SNP	Morales-Hojas *et al*., 2020
Migration and invasion	SSR	Delmotte *et al*., 2002 Guo *et al*., 2005 Liu *et al*., 2009 Angham *et al*., 2012
	mtDNA	De Barro and Ahmed 2011 Yang *et al*., 2012 Abdallah *et al*., 2013 Sun *et al*., 2020
	RFLP	Liu *et al*., 2010
	SNP	Whitfield *et al*., 2006
Mating reproduction mode	SSR	Sunnucks *et al*., 1996a Franck *et al*., 1999 Reddy *et al*., 1999 Hannonen *et al*., 2002 Lewis *et al*., 2002 Bretman *et al*., 2004 Hacker *et al*., 2005

1.4.4.1. Genetic relationship and phylogeny among populations

Application of molecular markers techniques in the identification of insects among different populations, has greatly advanced the research on insect phylogeny. Estoup et al. (1995) analyzed the genetic relationship between individuals in bee colonies using microsatellites, illustrating that *Apis mellifera* L.

evolved from three distantly related ancestors. England et al. (1996) used 8 pairs of microsatellite primers to analyze the population structure of *Drosophila melanogaster*, showing that microsatellites had higher polymorphisms than heterozygous enzymes. Fuller et al. (1999) used 7 pairs of microsatellite primers to conduct genetic studies on cotton aphid populations in greenhouses in several regions of France, showing a domination by obligate parthenogenesis. Tsutsui et al. (2001) used mitochondrial DNA and microsatellites to study the population origin of the Argentine ant, *Linepithema humile* (Mayr). Llewellyn et al. (2003) used 4 pairs of microsatellite primers to study the genetic structure of 4 geographical populations of *Sitobion avenae* for 2 years, and the results showed that the allele frequency was stable between populations and time. Tuda et al. (2004) used three mitochondrial genes to perform sequence analysis to reconstruct the phylogeny of the genus *Callosobruchus chinensis* in Asia and Africa. Hou et al. (2006) constructed fingerprints of 96 silkworm species with different origins, and discussed the origin and evolution of silkworm using microsatellites. Niu et al. (2006) used AFLP on beet armyworm (*Spodoptera exigua*) showing different regional geographic populations associated to frequent gene exchanges. Franck et al. (2010) also studied the genetic structure characteristics of *Cydia pomonella* populations by AFLP in different geographies. No geographical isolation among the populations in Switzerland, South Africa and Italy was observed. Based on a microsatellite analysis, Margaritopoulos et al. (2007) found that aphid clone selection and gene flow between sexual clones of *M. persicae* were two important factors that constitute the genetic structure of aphid populations. The two subspecies were widespread worldwide, indicating that world trade had a significant impact on their population structure. Mukha et al. (2007) analyzed the genetic variation and the genetic structure between populations of the German cockroach (*Blattella germanica*) using RFLP. High genetic variation and significant genetic structure were observed with no obvious correlation between the geographical distance and genetic structure. Wondji et al. (2007) analyzed 50 gene fragments of 21 stream *Anopheles funestus* by SNP, and finally detected a total of 494 SNPs, of which 303 were in the gene coding region (including 5 insertion/deletion sites), they had high polymorphism. Albre et al. (2008) used mitochondrial *COII* and *ND5* genes to study the molecular phylogeny of *Erebia tyndarus*. Eleven species that are difficult to identify in western of Europe were revealed. Shufran and Payton (2009) found no difference in *COI* sequence among different biotypes of wheat aphid. Liu et al. (2010) used 7 pairs of microsatellite primers to study and analyze the genetic differences of *M. persicae* from 13 geographic populations of Gansu. There were relatively few gene exchanges between them, are thought to be more prone to genetic drifting.

1.4.4.2. Defining the relationship among species

The application of molecular markers in genetic variation studies of geographic populations not only explores the classification of species from the population genetic perspectives, especially has unique advantages in the identification of sub-

species stages, but also reveal the speciation processes and evolutionary trend. Yang et al. (2008) amplified the 16S rDNA and identified 4 species of *Liposcelis* in China and the Czech Republic by using RFLP. Between them, 3 species were geographically isolated, providing a technical basis for the identification of different geographical sources. Teng et al. (2007) used 8 microsatellites to study the genetic structure of 25 Chinese *Locusta migratoria* populations (1381 individuals in total), and reclassified the sub-elements of Chinese species. Sun et al. (2012) studied the taxonomic status of *Laodelphax striatellus* red type and green type through microsatellites. Morales-Hojas et al. (2020) studied cereal aphids from China and UK by using SNP. The results showed that a superclone was now dominant across the geographic distribution in the UK with low genetic diversity. In China, *S. miscanthi* populations were mostly holocyclic with high genetic differentiation between geographic locations. The application of molecular marker technology provides an effective technical method for comprehensively understanding the evolutionary relationship of different taxonomic categories of insects.

1.4.4.3. Insect migration and invasion

Molecular markers have shown their unique characteristics in the study of insect migration. Researching the geographical distribution of genetic variation in insect populations could infer gene infiltration or reproductive isolation, providing molecular genetic evidence for the study of insect migration and spread. Delmotte et al. (2002) studied the population genetic structure of *Rhopalosiphum padi* with a combination of microsatellites and isoenzymes, and deduced that the migration distance of this aphid was 1000 km. Guo et al. (2005) studied the genetic structure of 15 geographical populations of Chinese wheat aphid (*Macrosiphum miscanthi* Takahashi) through 5 microsatellites, and found that the genetic distance of populations among eastern plain populations and western plateau was close, respectively. It was speculated that the greater the geographic distance, the greater the genetic distance. Whitfield et al. (2006) used single nucleotide diversity SNP analysis and found that the invasion of African honeybees to the lower-temperature Eurasian continent was the result of at least two hybridizations between African honeybees and European honeybees. Liu et al. (2008) used 13 polymorphic microsatellite loci to study the genetic diversity and genetic structure of 14 geographical populations of *Pectinophora gossypiella* in China and found that *P. gossypiella* was a multiple invasion from the United States and Pakistan to China, there was a significant differentiation among all the geographical populations. Angham et al. (2012) used 16 microsatellite loci to genotype 31 *Tetranychus evansi* populations around the world, and used the ABC (approximate bayesian computation) analysis method to reveal invasion pathway of *T. evansi* worldwide. Abdallah et al. (2013) used the mitochondrial *COI* gene to study the genetic diversity and population diffusion path of the Arabian rhinoceros beetle (*Oryctes agamemnon arabicus*) population. Populations of *O. agamemnon* had a low level of genetic variation, and the expansion of the worm in Tunisia was

mainly dependent on human activities. Sun et al. (2020) studied the genetic diversity and population diffusion paths of *S. miscanthi* populations in China using mitochondrial, endosymbiotic bacteria and nuclear genes. Populations of aphids had high haplotype diversity, and the origin of the aphids was in the south and southwest of China. Molecular markers show great advantages in revealing the invasion history and invasion paths of pests.

1.4.4.4. The mating and reproduction behavior of insects

In the research of insect ecology, molecular markers could be used for paternity identification of insects through molecular markers could reveal the mating and reproductive behavior of insects. Sunnucks et al. (1996a) used microsatellites to detect the genome of *S. miscanthi* and explained the genetic relationship between its periodic parthenogenesis. Franck et al. (1999) used microsatellites to study the mixure of sperm in the queen bee's seminal vesicles at different times after mating. Reddy et al. (1999) used SSR to study the silkworm genome, and used 15 microsatellite primers to analyze the polymorphisms of 13 strains of the silkworm, and identified the unique microsatellite marker of non-diapause and diapause strains. Hannonen et al. (2002) used SSR to study reproductive conflicts in ant colonies. Lewis et al. (2000) used 5 microsatellite loci to study the mating mechanism of *Pissodes strobi*, an important forest pest. Bretman et al. (2004) used three microsatellites to study the mating mechanism of the polyandry double-spotted cricket (*Gryllus bimaculatus*).

1.4.5. Conclusions

In the past 20 years, molecular marker technology has been widely used in insect research, providing valuable information for studying aphid's population diversity. With the development of biotechnology and information technology, such as the insect genome project, the development of microsatellites, and the emergence of Bayesian approximate estimation methods, more accurate analysis results and more potential application value were conducted in research on aphids. However, there are species specificity, time and space specificity during sample, which leads to differences in results. Therefore, multiple molecular marker technologies and high throughput sequencing should be combined to acquired accurate and reliable results. In addition, the sampling density and coverage, different identification of aphids or other agricultural pest sources and invasion routes are of great significance in control of invasive species. The source of invasive species can be used to assess the physiological adaptation and evolution of invasive species. Furthermore, molecular markers are also effective tools for exploring the mechanism of insect's invasion. It is assumed that molecular markers in the past were apparent cognitions. Nowadays, we can directly explore the functional genes that enable invasive insects to rapidly evolve and adapt to the environment.

1.5. Insect endosymbionts

1.5.1. Overview of insect endosymbionts

The relationship between two or more organisms interacting and living together is called symbiotic relationship, which exists widely in the biological world. When microbes live in symbiosis with other organisms, they are called symbionts. Insects are among the most abundant and diverse multicellular animals, with approximately one million species identified to date, and they play an important role in the biosphere. Insects comprise more than 75% of all livings, having an important impact on agriculture and human health (Foottit et al., 2009). Symbionts exist widely inside the body of insects. According to phylogenetic analysis, it is speculated that insects and bacteria began to interact and gradually formed symbiotic relationships as early as 250 million years ago (Moran et al., 1998). Almost all life activities of insect-host have the participation of microorganisms in the body (A. E. Douglas, 2009; Seth et al., 2019).Symbionts have played a major role in the adaptation and evolution of insects (Moya et al., 2008; Oliver et al., 2018). Symbiotic bacteria are closely related to the life activities of insects. Through long-term evolution, they have formed a mutually beneficial symbiosis relationship with insects. (McFall-Ngai et al., 2013; Angela E. Douglas, 2015). Most of the symbiotic bacteria in popular research are endosymbionts. Episymbiotic are symbionts that live outside the cells of host insects, such as intestinal microbes (Engel et al., 2013). Endosymbionts was defined in 1879 as a microbial species that lives and is closely related to one or more other species (Bary, 1879).

1.5.2. Distribution of endosymbionts in insects

Almost all insects have endosymbionts, which are widely distributed. At present, *Wolbachia*, the most widely distributed endosymbiont in insects, has a lot of hosts. Studies have shown that the infection rate of *Wolbachia* in Insecta is about 66%, including Homoptera, Diptera, Orthoptera, Hymenoptera, Lepidoptera (Werren et al., 1995). Endosymbionts are also widely distributed in many parts of insects. In addition to being distributed in the reproductive system, *Wolbachia* is also widely distributed in non-reproductive tissues such as the head, chest, abdomen, salivary glands, and digestive tract. During the embryonic development of *Drosophila*, *Wolbachia* is distributed in the surface cells and has no connection with fertilization (Rongo et al., 1996; Hadfield et al., 1999).

1.5.3. Classification of endosymbionts

Endosymbionts are mainly divided into primary and secondary symbionts. First ones are necessary for the host, mainly to provide the host with nutrients needed to maintain life activities. Secondary symbionts are facultative, related to the degree of host evolution, sometimes not necessary, and may even be harmful (D. Q. Chen et al., 1997).

1.5.4. Transmission routes of endosymbionts

Over the past two decades, there have been many studies on transmission routes of endosymbionts either vertically or horizontally. Most endosymbionts live obligately inside the host body and cannot survive independently. Transmission of endosymbionts between hosts is extremely important for their survival and function (Jiggins et al., 2011).

Vertical transmission is the most basic transmission route of symbionts. They infect eggs from host insects, which transfer themselves to off-springs (Hoffmann et al., 1990). Primary symbionts, *Buchnera* of aphids, *Portiera* of *Bemisia tabaci* and *YLS* of rice planthopper, *Sulcia* and *Nasuia* of *Nephotettix bipunctatus* are vertically transmitted between insects (Noda et al., 2003; Oliver et al., 2014; Wei et al., 2019). Secondary symbionts also could be vertically transmitted, but the vertical transmission efficiency of different symbionts is not the same (Hui et al., 2010).

Although most endosymbionts use vertical transmission as the main transmission route, horizontal transmission is also very common between endosymbionts in nature. Horizontal transmission has important implications for the proliferation of endosymbionts, the adaptability and evolution of the host (Henry et al., 2013). Host parasites and host feeding transmission are two ways of horizontal transmission of secondary symbionts. *Regiella insecticola*, *Hamiltonella defense* and *Arsenophonus sp*. could spread steadily horizontally via parasitic wasps (Duron et al., 2010; Gonella et al., 2012). *Scaphoideus titanus* spreads horizontally *Cardinium* and *Asaiaby* by feeding on grape leaves (Gonella et al., 2012). The horizontal transmission of secondary symbionts exists within and between species, which expands the host range of endosymbionts.

1.5.5. The function of symbionts in insects

1.5.5.1 Providing nutrition and material metabolism

Because many insects cannot obtain the nutrients necessary for growth and development from a single food, they rely on the essential nutrients provided by their endosymbionts to solve the problem of unbalanced intake of nutrients (Munson et al., 1991; Ishikawa, 2000; Wilkinson et al., 2001; Hosokawa et al., 2010;; Liang et al., 2018). The endosymbiont in the rice pest *Nilaparvata lugens* used the metabolic waste of the host to synthesize essential amino acids for the growth and development of the brown planthopper (Sasaki et al., 1996). *Sulcia* symbiont derived from a leafhopper that feeds on the xylem sap of woody plants, provides essential amino acids for host insects (Mccutcheon et al., 2009). *Rhodococcus rhodnii*, a symbiont of *Rhodnius prolixus*, provides vitamin B to the host (Eichler et al., 2002). *Cardinium*, a symbiont of *Encarsia pergandiella*, also provides biotin to the host (Penz et al., 2012).

1.5.5.2. Regulation of host reproduction

Symbionts infect host insects through vertical or horizontal transmission, and play a vital role in the growth, development and reproduction of host insects.

Asaia is one kind of mosquito symbionts that promotes the development of larvae of *Anopheles stephensi* and *A. gambiae* (Chouaia et al., 2012; Mitraka et al., 2013). Insect endosymbionts could also indirectly affect the reproductive ability of host insects by changing the fitness of plants (Himler et al., 2011). Multiple infections of symbionts in *Tetranychus cinnabarinus* can significantly improve the host's oviposition and fitness (Y.-K. Zhang et al., 2018).

1.5.5.3. Regulation of host fitness

External uncertain factors affect insects in nature, such as temperature, insecticides and pesticides. Host insects can tolerate the effects of adverse environment with the assistance of the endosymbionts. *Rickettsia* in the *Bemisia tabaci* expressed heat-resistant molecules and was evenly distributed in the *B. tabaci* to improve the heat resistance (Brumin et al., 2011). The endosymbiont of *Oryzaephilus surinamensis* could also increase the host's fitness in a dry environment by affecting the thickness of the host's stratum corneum (Engl et al., 2017). There were more *Wolbachia* in the organophosphate-resistant strain *Culex mosquitoes* than the sensitive strain (Echaubard et al., 2010). The endosymbionts of the same biotype of *Bemisia tabaci* would vary according to the host plant and geographical location (Cass et al., 2016). But not all symbionts have a beneficial effect on the host. For example, the symbiotic bacteria in the bark beetle larvae can hinder the growth of the larvae by breaking down saccharides (B. Wang et al., 2012).

1.5.5.4. Enhancing host ability to defend against natural enemies and pathogenic microbes

Insects need to face various survival pressures in nature. Numerous studies demonstrated that symbiotic bacteria can protect host insects from predation and parasitism by natural enemies, and resist pathogenic microbes. Methane was produced by termite endosymbionts, which effectively prevented predation by their natural enemies (Rasmussen et al., 1983). *Spiroplasma* in fruit flies can improve resistance to parasitic nematodes and wasps (Jaenike et al., 2010; Xie et al., 2010).

Nowadays, many insect endosymbionts have been found to be related to the resistance to pathogenic microbes. However, the mechanism by which endosymbionts enhance host resistance is still unclear. Termite endosymbionts could defend against the invasion of alien pathogenic microbes (Oliver et al., 2003) while *Wolbachia* enhanced resistance of fruit flies to RNA viruses (Glaser et al., 2010). Also, *Streptomyces* could provide effective antibacterial defense to the wasp *Philanthus* larvae by producing antibiotics (Kroiss et al., 2010). *Candidatus* is a symbiont that can secrete a mixture of 9 antibiotics in the wasp, which effectively prevents pathogenic microbes from infecting the wasp. Similarly, *Rickettsia* in *Bemisia tabaci* contribute to the resistance in the invasion of pathogenic microbes (Hendry et al., 2014).

1.5.6. Aphid endosymbionts

Aphids, like other insects, have two types of endosymbionts, which are divided into primary and secondary symbionts according to their effects on the host. The 16SrDNA sequence analysis of the primary endosymbiont of aphids and other bacteria revealed that *Buchnera aphidicola*, the primary symbiont of aphids, was found (Baumann et al., 1995). *B. aphidicola* exists in the bacteriocyte of all aphids and forms a stable co-evolutionary relationship with host insects. It spreads vertically between the mother and offspring of aphids through the ovaries (Baumann, 2005). It not only provides nutrients such as essential amino acids for aphids, but also anabolic enzymes that make aphids quickly adapt to new host plants; its infection type and simple variability of genetic background are conducive to the formation of aphid's biotypes (B. C. Campbell, 1990).

The secondary symbionts found in aphids are mostly detected in pea aphid, including *Serratia symbiotica* (Fukatsu et al., 2000), *Hamiltonella defensa* (PABS) (Darby, 2001), *Regiella insecticola* (PAUS) (T. Tsuchida, Koga, Shibao, et al., 2010), *Rickettsia* sp. (PAR) (D. Q. Chen et al., 1996), *Spiroplasma* sp. (Fukatsu et al., 2001), *Wolbachia pipientis* (Jeyaprakash et al., 2010) and *Arsenophonus* sp. (Russell et al., 2010). Recent studies have found that secondary symbionts are mainly indirectly related to the fitness of the host aphids, the ability to resist natural enemies, the resistance to fungal infections and other characteristics.

S. symbiotica could enhance the tolerance of aphids to heat stress in pea aphid (Montllor et al., 2010) and improve the ability to resist to parasitic wasps until a 30% reduction rate (Gil-Turnes et al., 1992). *H. defensa* in the pea aphid was found to induce resistance in parasitic wasps (Ferrari et al., 2010). After *Euonymus vulgaris* was infected with *H. defensa*, the parasitic rate of the *Lysiphlebus fabarum* was greatly reduced (Rossbacher et al., 2020). During the adaptation process of aphids and host plants, the genetic variability of symbiotic bacteria makes their genetic composition easy to change. When studying the genetic differentiation of pea aphid feeding on different host plants, it was found that secondary symbionts have a certain relationship with the host fitness and population differentiation of pea aphid (Simon et al., 2003). The latter infected with *S. symbiotica* had increased sensitivity to pesticides (Skaljac et al., 2018). *R. insecticola* could expand the species of plants that pea aphid feed on (Tsuchida et al., 2004). It could also determine whether the pea aphid could live on alfalfa (T. Tsuchida et al., 2011). *Rickettsiella*, a symbiont of *A. pisum*, induced the host insect's body color to change from red to green to avoid being preyed by ladybugs that like to prey on red aphids. Another symbiont, *Serratia*, could assist the pea aphid to escape the parasitism from parasitic wasps (T. Tsuchida, Koga, Horikawa, et al., 2010).

1.5.7. Effect of aphid endosymbionts on population differentiation

Insect bacteriocyte and associated endosymbionts are the best system for studying the interaction between prokaryotic genome and eukaryotic genome in one single cell. The system is also very valuable for studying the origin of cellular organic matter. At the same time, the evolutionary relationship between endosymbionts and their hosts is a useful tool for studying the phylogeny and biodiversity of aphids. The consistency of the evolutionary relationship between the primary endosymbionts and the host, the genetic diversity of the secondary endosymbionts and the inconsistency of the phylogeny with the host, all of those are good evidences for studying the phylogeny of aphids and their biodiversity (Thao et al., 2000; Sandstrm et al., 2001).

The parallel and consistent phylogeny of aphids and primary endosymbionts may reflect their co-evolutionary relationship (J. F. Douglas, 2005). In addition, the endosymbiont is inherited to the offspring by maternal inheritance, so that the symbionts could reflect the evolutionary history of the host aphid to a certain extent (Symula et al., 2011). The phylogeny of *trpB* gene from the aphid *Buchnera* was discussed (J. J. Wernegreen, 2002). The study of the phylogeny between the host and the endosymbiont is helpful to better understand the co-evolutionary history of the host and the endosymbionts (Nieberding et al., 2007).

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2

Chapter II: Effects of different temperatures on the development and reproduction of *Sitobion miscanthi* from six different regions in China

From **Sun, J**., Tan, X., Li, Q., Francis, F., Chen, J. Effects of different temperatures on the development and reproduction of *Sitobion miscanthi* from six different regions in China. Submitted in *Frontiers in Ecology and Evolution.*

2.1. Foreword

In this work, we performed life history traits of six populations of *Sitobion miscanthi* in China at different temperatures to determine the effects of temperature and associated latitude on aphids. Previous literature reports indicated that the "the hotter the better" hypothesis, the higher the optimal temperature, the higher the maximum thermal performance of the organism should be. Therefore, we assume that the aphid also follows this assumption.

Optimal temperature of *S. miscanthi* was around 22°C. We adjusted the temperature up and down by 5°C and set three levels. The experiment was selected from 6 populations in the main wheat regions of China. Under constant temperature, we collected their life parameters (development time, adult longevity, fecundity, etc.). Analyzing and discussing the results, it was found that contradicting the original hypothesis, the adult longevity and fecundity of aphids did not increase with higher temperatures. We speculated that this result is most likely related to the migration of aphids.

2.2. Abstract

Rising temperature caused by global warming have a high impact on plant growth and pest population dynamics worldwide especially wheat aphids. In this study, *Sitobion miscanthi* individuals from six geographic populations located in the different major wheat-producing areas in China, were compared with regard to their growth, development, survival, and reproductive indexes under the different temperature conditions (17, 22 and 27°C). A population life table analysis and a correlation analysis between geographic factors and *S. miscanthi* longevity were also performed. The aphid nymphal developmental duration and adult longevity of all six geographic populations gradually decreased with increasing temperature, except the Yinchuan population that increased at 27°C. There were no significant differences in fecundity among the six populations at 17°C, while southern populations were significantly different at 27°C: the fecundity of Suzhou and Kunming were significantly higher than that in Wuhan. The fecundity of southern populations was no significant difference with northern populations at 17°C. The fecundity of southern populations was significantly lower than that of northern populations at 27°C. There were no significant differences of the fecundity among the northern populations. In addition, there was a positive correlation between latitude and aphid longevity. These findings are somewhat surprising given the fact that other research showed the hypothesis of “the hotter the better”. The possible interference resulted from the aphid migration. These results will be useful for predicting potential aphid outbreak in China.

Keywords

Sitobion miscanthi; population; longevity; temperature; development, reproduction

2.3. Introduction

Wheat is a world major crop in temperate regions of the world (Kirkegaard et al., 2008; Nirmal et al., 2017). The main production bases for high-quality wheat in China are located on the Yellow–Huai–Hai Plain and the North China Plain (Jin et al., 2021). Aphids cause severe crop yield reductions and result in significant economic losses each year (D. H. Wang et al., 2014). *Sitobion miscanthi* (Takahashi) (Hemiptera: Aphididae) as a major insect pest in temperate regions, has a short development cycle and a high reproductive rate (Raychaudhuri et al., 1973; Turak et al., 1998; Hawkes et al., 2005; Singh et al., 2009; Z. Wang et al., 2009; Hales et al., 2010; J. Chen et al., 2011). *S. miscanthi* is anholocyclic in most geographic areas. In rare cases there are sexual forms: an oviparae (sexual female) collected on Polygonum chinense in India may be *S. miscanthi* (David, 1975). In Japan and Korea, *Sitobion akebiae* may be a synonym, although *S. akebiae* is holocyclic (lays overwintering eggs). But the taxonomy of the *miscanthi* and *akebiae* group requires further clarification. The evidence for holocycly need more investigations (Blackman et al., 1984). Temperature is an important abiotic factor that affects aphid population and biological parameters including insect growth and development (Osawa, 1993; Nyaanga et al., 2005; Del et al., 2013; Zhu et al., 2017). Optimal growth rate and development cycle of aphids require adapted temperature range (Zhang et al., 2017; Bernard et al., 2018). For example, for codling moth the optimal range is between 10 and 30 ℃ (Rock et al., 1983). Also, the optimal range for egg, larval and egg-to-adult development of *Spodoptera frugiperda* recorded is between 26 and 32 ℃ (Plessis et al., 2020). Under extremely harsh natural conditions, aphids will initiate diapause adaptation strategies (Denlinger, 2002; Kroschel et al., 2013; Gang et al., 2016). In ectotherms, environmental temperatures influence the organism's biochemical reactions , with direct consequences for life history traits (Hochachka et al., 2002). Some studies show a correlation between the optimal thermal value and the mean temperature of locations by latitudinal or geographical analysis at intra- and inter-specific levels (Angilletta, 2009).

Environmental temperature affects the function and adaptability of ectotherms, revealing that the distribution of the organisms is mainly limited by their thermal fitness (Huey et al., 1993; Angilletta, 2009). Studying how ectotherms adapt to different thermal environments is particularly important to understand the changes in the life history (Clarke, 1993). "The hotter the better" hypothesis shows that the higher the optimal temperature is, the higher the maximum thermal performance of the organism should be (Huey et al., 1989). The two hypotheses are slightly different, but both predict that optimal thermal performance will achieve maximum performance at the most frequently experienced body temperature. Therefore, the genotype of the hot environment has a higher thermal optimization than that of the cold environment.

The fitness cost investigated included life histories (developmental time, fecundity, fertility and population growth), metabolism and behavior. Among them, life-table analyses are a means of determining the population characteristics

that predict population growth and describing developmental characteristics such as reproductive rates and life expectancies in a pest population (Davison et al., 2010; Hajar et al.; Liao et al., 2017). The effect of temperature on aphids has a lot reports under laboratory conditions on some host plants. The optimum temperature for the growth and development of grain aphid, *Sitobion avenae* is 15-25 ℃, at which it has the highest intrinsic growth rate (r_m) (Ahn et al., 2020). The highest r_m and finite rate of increase in *Acyrthosiphon pisum* (Harris) were observed at 25 ℃ (Luis et al., 2001). Some studies reported the temperature affect the aphid's behavior and biochemical parameters (Ma et al., 2012; Chen et al., 2013). However, few studies involved the effects of multiple factors such as temperature and latitude on aphid biology and ecology.

Given that temperature and geographic factors may play an important role in the evolution of insects, measuring the life history traits of different populations under a series of developmental temperatures can reveal the adaptation of traits to general temperature conditions. In an effort to estimate the influence of the net effects of changing scenarios of climate, the positive and negative effects of temperature changes on insects need to be considered. Elevated temperature will bring them closer to their physiological optimal state, insects at high latitudes may have a higher adaptability (Kingsolver, 2009). The impact on populations in low latitudes is little known (C. A. Deutsch et al., 2008; Stange et al., 2010). Insects in low latitudes may suffer a decline in growth, fecundity and fitness in summer.

The aphids were exposed to three constant temperatures (17, 22 and 27 ℃). The optimal temperature of *S. miscanthi* is about 22℃, and the other two temperatures are increased and decreased by 5℃ respectively at this temperature value, making it within the range of the optimum temperature threshold for the aphid. We predict that the longevity and fecundity of *S. miscanthi* will decrease with increasing temperature. "Based on the hypothesis that higher temperature will eventually exceed its physiological optimal state, we predict that the longevity and fecundity of *S. miscanthi* will decrease with the increase of temperature. Therefore, in this study, the effects of three constant temperatures (17, 22 and 27 ℃) on the development and reproduction of different geographic populations of *S. miscanthi* were evaluated. It is expected to help for areawide predicting aphid outbreaks.

2.4. Materials and methods

2.4.1. Aphid colony

Aphid populations were collected from wheat plants in six major wheat-producing areas namely Suzhou (SZ), Wuhan (WH), Kunming (KM), Tai'an (TA), Langfang (LF) and Yinchuan (YC) in China by five-point sampling method from February to April 2018. The aphids were brought back to the laboratory and transferred to wheat seedlings. New born nymphal aphids from adults were transferred to new aphid-free wheat (cutivar, *Hengguan 35*) seedlings to continue feeding. The aphids performed 3-5 generations under lab conditions and were stably reproducing in order to achieve clonal homogeneity. Plants were grown in

controlled environmental chambers at 22 ± 1°C, 75 ± 5% relative humidity (RH), and a 16h photoperiod under a light intensity of 9000 lx.

2.4.2. Wheat plants

The wheat cultivar *Hengguan* 35 was used as host plant for aphid rearing. Wheat seedlings were updated every two weeks to maintain aphid reproduction. Nutrient soil and loess were sterilized, mixed with vermiculite at a ratio of 2:1:1 and placed in pots. Ten full-grain wheat seeds were then sown in each pot (20 cm in diameter). When the wheat plants had grown to 5 cm, aphids were inoculated to five vigorous seedlings. Temperature and humidity conditions were similar to those related to aphid rearing.

2.4.3. Experimental conditions

Aphids were reared on wheat leaves in three artificial climate rooms at 17, 22 and 27 °C, and keeping previous relative humidity and photoperiod conditions. One adult aphid was individually placed onto a wheat leaf at two-leaf stage and covered with a microcage. Each treatment included five pots, and four adult aphids were inoculated to each pot. Each treatment was repeated three times. After the nymphs were born, the adults were removed and all nymphs in each pot were at the same developmental stage.

The aphids were observed twice at 8:00 and 20:00 every day. The number and instars of nymphs as well as the developmental duration of each instar were recorded. The number of aphids produced was recorded until all adults had died.

2.4.4. Data analysis

Nymphal development duration, adult longevity, aphid longevity, fecundity was analyzed using ANOVA. The linear correlation analysis was used between the latitudes of six places and the fecundity of *S. miscanthi*. Two-way ANOVA was used to analyze the effects of temperature and latitude on the nymphal development duration, adult longevity, aphid longevity, fecundity of *S. miscanthi*. The experimental data were expressed as mean ± standard error (SE). Duncan's new multiple range test at $P < 0.05$ was used to compare the means and determine the significance of differences between variables. To assess age-specific reproduction, we used life-table analysis. *lx* was survivorship of the original cohort over age interval from day *x-1* to day *x*. *mx* was the mean number of female offspring produced per surviving female during the age interval *x*. The reproductive rate (R_0) and the intrinsic rate of population increase(r_m) was calculated by using the Euler-Lotka equation (Aline et al., 2000). R_0, r_m, the generation average period (T), the weekly growth rate (λ), and the population doubling time (D_t) were calculated (Suleman et al., 1979; Wei et al., 2018). The data were analyzed using SPSS statistical software, version 26.0 (SPSS, Inc., Chicago, IL, USA). Graphs were made using the GraphPad Prism 8 biostatistics software (GraphPad Software, USA).

2.5. Results

2.5.1. Distribution of sampling locations and their basic information

Northern and southern regions of China were divided by the Qinling Mountains and the Huaihe River. Insect samples were obtained from six populations located in the main wheat-producing areas of China, in which three (SZ, WH and KM) belong to southern populations and the other three (TA, LF and YC) northern populations. The sample information included the names of the collection places, latitudes and mean annual temperature were showed in the Figure 2-1.

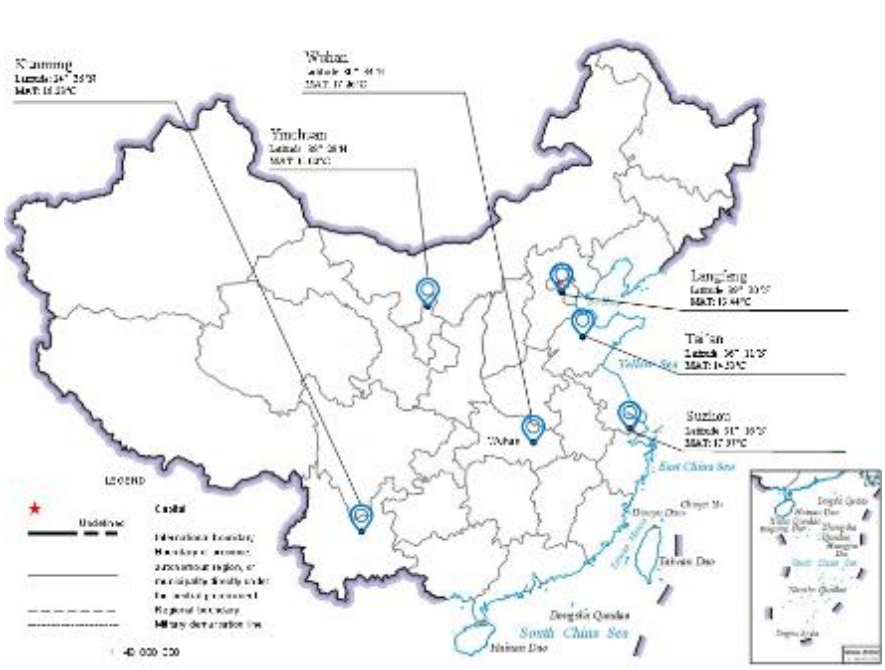


Figure 2-1. Map of China showing the locations of the sampling sites and the mean annual temperature (MAT) were calculated on 2015 and 2018 period.

2.5.2. Effect of temperatures and latitude on nymphal development duration of Sitobion miscanthi

Temperature and latitude significantly affected nymphal development duration (NDD) of aphids (Table 2-1). There were significant differences in developmental time at different temperatures (Table 2-2).

Table 2-1. Effects of temperature and latitude on the nymphal development duration, adult longevity, longevity and fecundity of *Sitobion miscanthi*

Variable	Source	*df*	*F*	*P*
Nymphal development duration(D)	Temperature	2	45.61	< 0.001
	Latitude	5	39.74	< 0.001
	Temperature*Latitude	10	4.14	< 0.001
Adult longevity(D)	Temperature	2	114.62	< 0.001
	Latitude	5	62.35	< 0.001
	Temperature*Latitude	10	9.61	< 0.001
Longevity(D)	Temperature	2	235.05	< 0.001
	Latitude	5	35.35	< 0.001
	Temperature*Latitude	10	7.42	< 0.001
Fecundity	Temperature	2	107.6	< 0.001
	Latitude	5	2.31	0.044
	Temperature*Latitude	10	9.81	< 0.001

[1] Temperatures: 17, 22 and 27°C; 2 Latitudes: 24.59°N (Kunming), 30.58°N (Wuhan), 31.3°N (Suzhou), 36.19°N (Tai'an), 38.47°N (Yinchuan), 39.5°N (Langfang).

Table 2-2. Nymphal development duration (D) of the wheat aphid, *Sitobion miscanthi*, at 17, 22 and 27°C.

TEMP (°C)	Populations	Development time (Mean ± SE)	*F*	*P*
17	SZ	13.89 ± 0.32	18.73	< 0.001
	WH	13.51 ± 0.36		
	KM	12.67 ± 0.28		
	TA	11.44 ± 0.22		
	LF	11.06 ± 0.13		
	YC	11.61 ± 0.25		
22	SZ	12.28 ± 0.34	2.41	0.042
	WH	12.33 ± 0.20		
	KM	11.39 ± 0.23		
	TA	11.22 ± 0.34		
	LF	11.44 ± 0.32		
	YC	11.27 ± 0.35		
	SZ	12.28 ± 0.34		
	WH	12.5 ± 0.30		

27	KM	11.33 ± 0.11	37.43	< 0.001
	TA	10.06 ± 0.01		
	LF	9.83 ± 0.90		
	YC	9.72 ± 0.11		

The nymphal development duration of the northern population was significantly shorter at 17 and 27°C than that at 22°C (Figure 2-2). Within the southern populations, the nymphal development duration of SZ and WH were significantly longer than that of KM at 17 and 27°C. However, the duration was almost the same at 22°C.

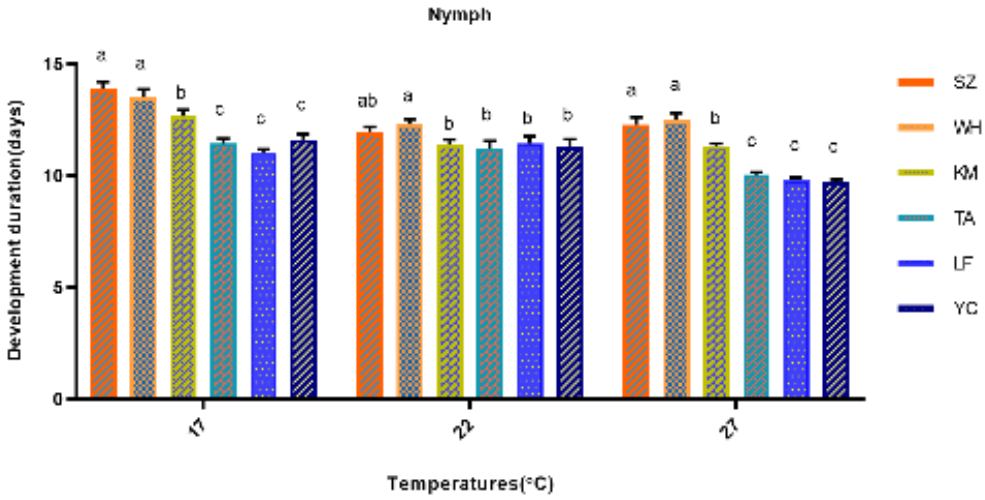


Figure 2-2. Effect of temperatures and latitude on nymphal development duration of *Sitobion miscanthi*. Nymphal development duration at different temperatures.

The nymphal development duration in the southern population declined at 22°C, while the northern population did that at 27°C.

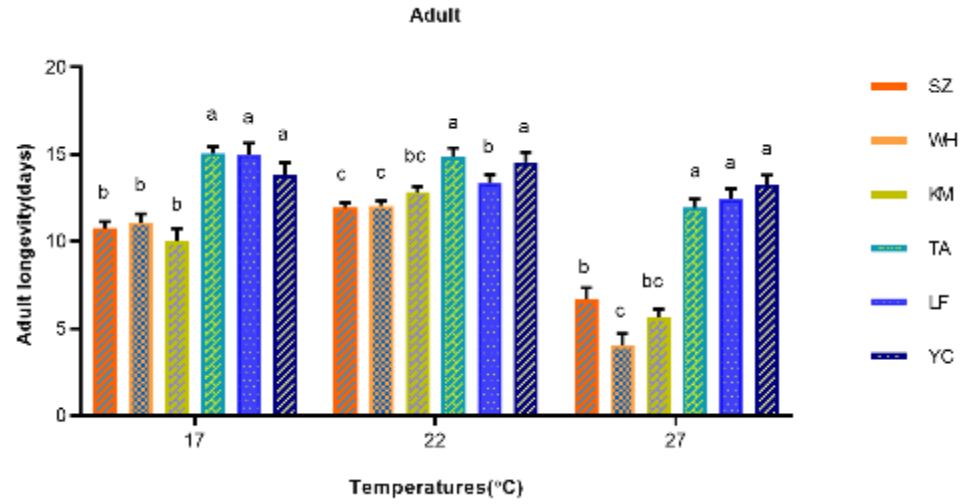
2.5.3. Effect of temperatures and latitude on adult longevity of Sitobion miscanthi

Temperature and latitude significantly affected adult longevity of aphids (Table 1). There were significant differences in adult longevity under different temperature conditions (Table 2-3).

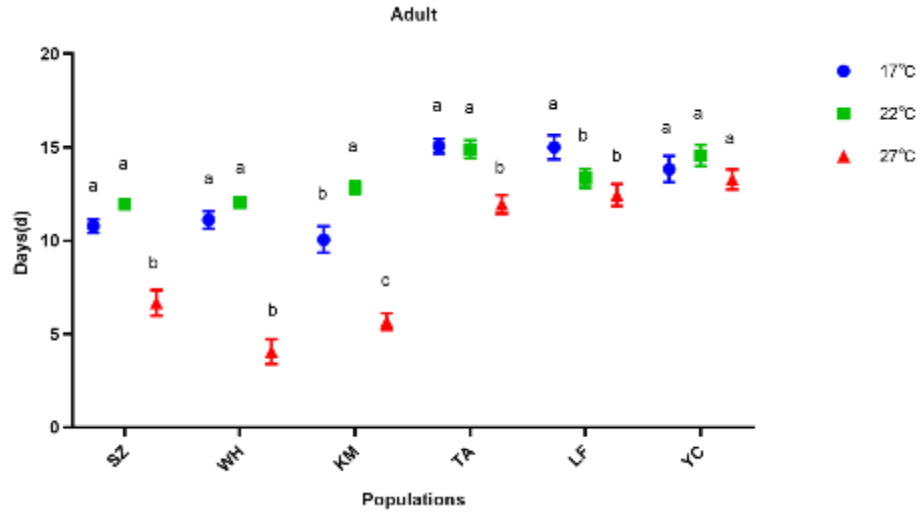
Table 2-3. Adult longevity (day) of wheat aphid, *Sitobion miscanthi*, at 17, 22 and 27°C;

TEMP (°C)	Populations	Adult longevity (Mean ± SE)	*F*	*P*
17	SZ	10.78 ± 0.37	15.83	< 0.001
	WH	11.11 ± 0.48		
	KM	10.06 ± 0.71		
	TA	15.05 ± 0.39		
	LF	15.01 ± 0.64		
	YC	13.83 ± 0.70		
22	SZ	11.94 ± 0.30	8.55	< 0.001
	WH	12.06 ± 0.27		
	KM	12.83 ± 0.33		
	TA	14.89 ± 0.48		
	LF	13.33 ± 0.50		
	YC	14.56 ± 0.57		
27	SZ	6.67 ± 0.68	48.38	< 0.001
	WH	4.06 ± 0.66		
	KM	5.67 ± 0.44		
	TA	11.94 ± 0.50		
	LF	12.44 ± 0.59		
	YC	13.28 ± 0.54		

The adult longevities in the southern populations were shorter than those of northern populations (Figure 2-3). Within the northern populations, there was no significantly difference in the adult longevity at 17 and 27°C.



(a)



(b)

Figure 2-3. Effect of temperatures and latitude on adult longevity of *Sitobion miscanthi*. (a) Adult longevity at different temperatures. (b) Adult longevity among different populations.

The adult longevity from the southern population was all decreased at 27°C, while there was not obvious difference between the northern and the southern

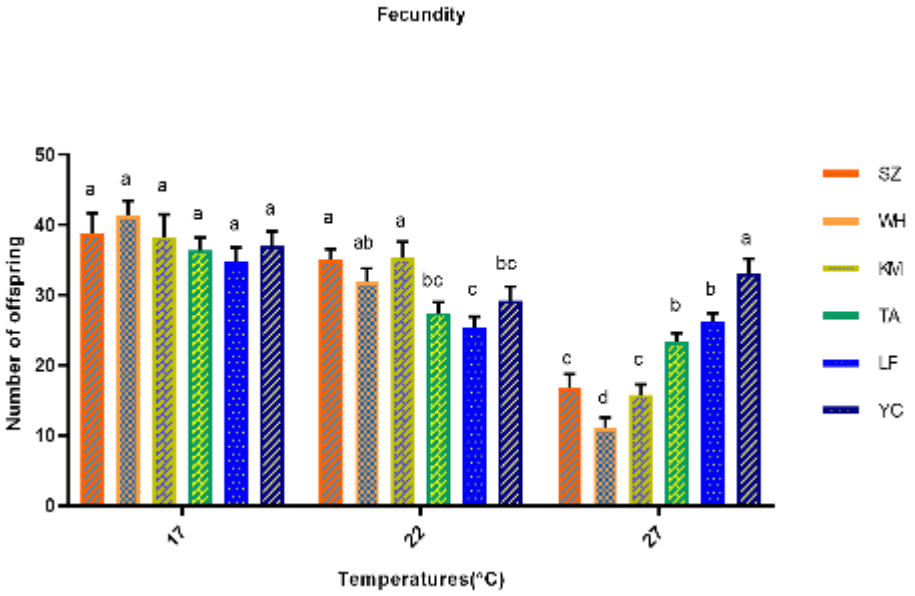
populations.

2.5.4. Effect of temperatures and latitude on fecundity of Sitobion miscanthi

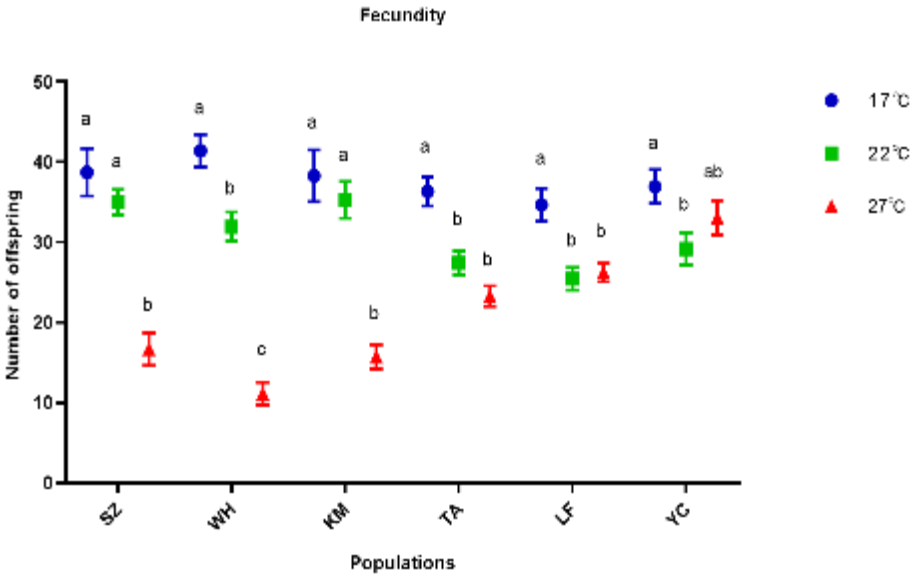
Aphid fecundity was significantly affected by temperature and latitude (Table 1). Compared to effect of latitude on aphid fecundity, a larger impact on aphid fecundity was observed in temperature. The fecundity at 22 and 27°C were significantly higher than that at 17°C (Table 2-4). The fecundity of southern populations was significantly lower than that of northern populations at 27°C. The fecundity of the southern population declined significantly at 27°C, while the northern population did at 22°C (Figure 2-4).

Table 2-4. Fecundity of wheat aphid, *Sitobion miscanthi*, at 17, 22 and 27°C.

TEMP (°C)	Populations	Offspring Per adult (Mean ± SE)	*F*	*P*
	SZ	38.72 ± 2.96		
	WH	41.06 ± 2.07		
17	KM	38.28 ± 3.22	0.91	0.479
	TA	36.33 ± 1.81		
	LF	34.67 ± 2.05		
	YC	36.94 ± 2.10		
	SZ	35.00 ± 1.58		
	WH	31.94 ± 1.84		
22	KM	35.28 ± 2.29	5.04	< 0.001
	TA	27.44 ± 1.50		
	LF	25.44 ± 1.43		
	YC	29.17 ± 2.01		
	SZ	16.67 ± 2.04		
	WH	11.11 ± 1.43		
27	KM	15.72 ± 1.52	24.08	< 0.001
	TA	23.28 ± 1.28		
	LF	26.22 ± 1.16		
	YC	33.06 ± 2.14		


Fecundity
Number of offspring
Temperatures(°C)
17
22
27
SZ
WH
KM
TA
LF
YC

(a)

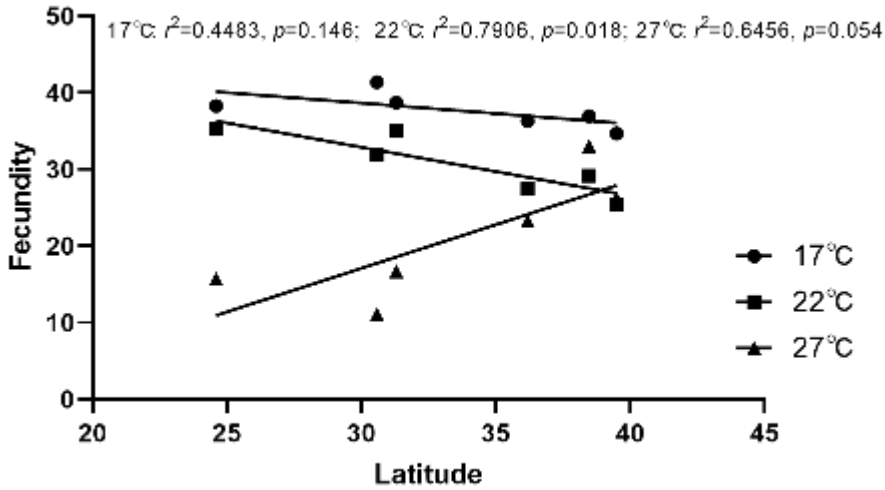

Fecundity
Number of offspring
Populations
SZ
WH
KM
TA
LF
YC
17°C
22°C
27°C

(b)

Figure 2-4. Effect of temperatures and latitude on the fecundity of *Sitobion miscanthi*. (a) Fecundity of *Sitobion miscanthi* at different temperatures, (b) Fecundity of *Sitobion miscanthi* among different populations.

The correlation of aphid fecundity with latitude at different treated temperatures were analyzed and showed (Figure 2-5a). Based on data of the correlation coefficient, the fecundity and latitude of the aphids was significant negative at 22°C (r^2 = 0.7906, p = 0.018 <0.05), with the latitude increasing, aphid fecundity decreased. But fecundity and latitude had no statistical significance at 17°C (r^2 = 0.4483, p=0.146 >0.05) and 27°C (r^2 = 0.6456, p= 0.054 >0.05).

The correlation of aphid longevity with latitude at different treated temperatures were analyzed and showed in (Figure 2-5b). Based on data of the correlation coefficient, the longevity and latitude of the aphids was significant positive at 17°C (r^2 = 0.8389, p = 0.010 <0.05) and 27°C (r^2 = 0.8271, p = 0.012 <0.05), with the latitude increasing, aphid longevity raised. But longevity and latitude had no statistical significance at 22°C (r^2 = 0.4545, p = 0.142 > 0.05).



(a)

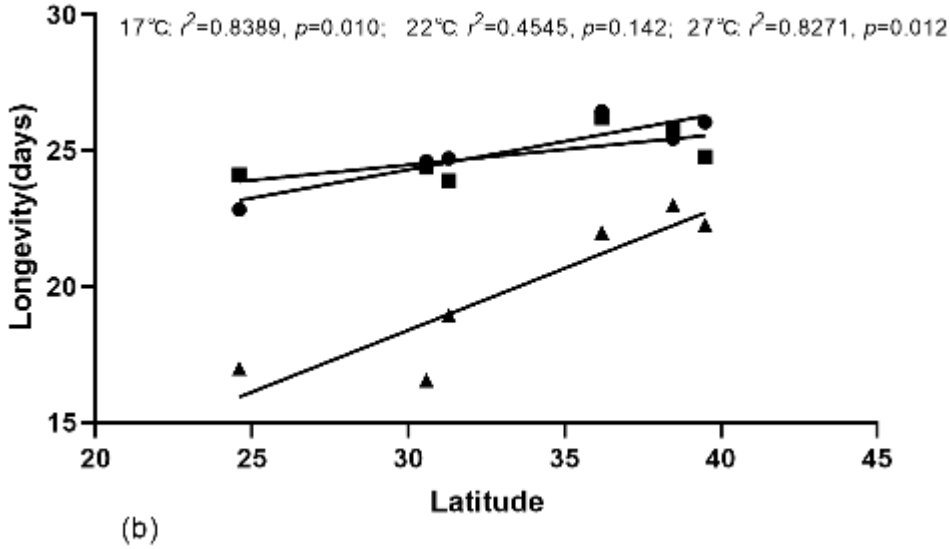


Figure 2-5. Analysis of the linear correlations between the latitudes of the six locations and the fecundity of *Sitobion miscanthi*. (a) Linear correlation between latitude and fecundity, (b) Linear correlation between latitude and Longevity.

2.5.5. Different temperatures on the life-table parameters of Sitobion miscanthi

The r_m values were higher in southern than in northern populations at 17 and 22°C, but lower at 27°C (Table 2-5). At 22°C, the r_m of the southern population was high (SZ: 0.22 ± 0.001; WH: 0.20 ± 0.001; KM: 0.22 ± 0.001). At 27°C, the rm of the northern population was high (TA: 0.21 ± 0.001; LF: 0.22 ± 0.001; YC: 0.23 ± 0.001). The R0 value of the southern populations was lower than that of the northern populations at 27°C. There was not significant different between southern and northern populations at 17°C. The λ values of populations were not significantly different.

Table 2-5. Life-table parameters of *Sitobion miscanthi* at each location at different temperatures.

TEMP（°）	Location	R_0 (Mean ± SE)	T (Mean ± SE)	r_m (Mean ± SE)	λ (Mean ± SE)
17	SZ	38.72±0.182b	18.73±0.022a	0.20±0.001f	1.22±0.001f
	WH	41.39±0.049a	18.32±0.008b	0.20±0.001c	1.23±0.001c
	KM	38.28±0.067c	17.18±0.008e	0.21±0.001b	1.24±0.001b
	TA	36.33±0.081e	18.04±0.045c	0.20±0.001e	1.22±0.001e
	LF	34.67±0.096f	17.58±0.018d	0.20±0.001d	1.22±0.001d
	YC	36.94±0.121d	16.91±0.037f	0.21±0.001a	1.24±0.001a

	SZ	35.00±0.081a	16.15±0.004d	0.22±0.001b	1.25±0.001b
	WH	31.94±0.145b	17.06±0.040c	0.20±0.001c	1.23±0.001c
22	KM	35.28±0.236a	15.96±0.010e	0.22±0.001a	1.25±0.001a
	TA	27.44±0.032d	17.59±0.050b	0.19±0.001e	1.21±0.001e
	LF	25.44±0.081e	17.89±0.053a	0.18±0.001f	1.20±0.001f
	YC	29.17±0.067c	17.49±0.044b	0.19±0.001d	1.21±0.001d
	SZ	16.67±0.049d	14.84±0.052b	0.19±0.001d	1.21±0.001d
	WH	11.11±0.067f	13.67±0.070c	0.18±0.001e	1.19±0.001e
27	KM	15.72±0.085e	13.08±0.061d	0.21±0.001c	1.24±0.001c
	TA	23.28±0.103c	14.77±0.029b	0.21±0.001b	1.24±0.001b
	LF	26.22±0.067b	14.62±0.032b	0.22±0.001a	1.25±0.001a
	YC	33.06±0.081a	15.44±0.088a	0.23±0.001a	1.25±0.001a

Note: R_0: The reproductive rate; r_m: the intrinsic rate of population increase; T: the generation average period; λ: the weekly growth rate.

2.6. Discussion

Temperature is a key variable that determines insect developmental duration, fecundity and population growth, as well as the physiological state of the poikilothermic organisms (Dean, 1974; Curtis A. Deutsch et al., 2008). Relevant research showed the influence of temperature on insect growth and reproduction. The optimal temperature for growth of *A. gossypii* and *S. avenae* were 24 ℃ (Gao et al., 2013) and 22.5 ℃ (Lykouressis, 1985) respectively. The optimum reproduction temperature of *Metopolophium dirhodum* was 25 ℃ (Zhou et al., 1992) while *Macrosiphum rosae* life cycle was optimized at 22 ℃ (Mehrparvar et al., 2007). In this study, the relationship between the development duration of *S. miscanthi* and the environmental temperature was not linear. Development of northern populations at 22 ℃ decreased significantly while southern populations decreased at 27 ℃. Incubators were used to simulate different temperatures and to explore their influence on the growth and development. We comprehensively evaluated the impact of different temperatures on the aphid population dynamics according to the experimental temperatures and the latitudes of the different collection locations. Our results showed that the change in environmental temperature had a certain influence on the growth development, fecundity, and intrinsic growth rate depending on the aphid population origin.

The correlation analyses in this study showed that temperature change significantly affected the development period of wheat aphids. Within a suitable temperature range for growth, as the temperature increases, the development period of wheat aphids were reduced, and the reproduction period was extended. Rising temperatures also changed the life-table parameters of the insects. Developmental velocity of *Laodelphax striatellus* was linearly related to

temperature between 15 and 25 ℃. At 30 or 32 ℃, the relationship was no longer linear and variation increased (Hachiya, 1990). Temperatures higher than 32 ℃ caused a decrease in developmental rate of *Aphis spiraecola* (J. J. Wang et al., 2000). In this study, with the increase in temperature, the duration and fecundity of the nymph and adult of the southern and northern populations were decreased. The declines in the aphid fecundity from both geographical origins of were different: the fecundity of the northern populations began to decline at 22 ℃, while that of the southern ones did not begin to decline until 27 ℃. Therefore, the fecundity of northern populations was more sensitive to temperature than that of southern populations. We compared the southern and northern populations, the nymphal development duration was longer; the adult longevity was shorter, the aphid longevity was shorter, and the overall adaptability was worse at 17 and 22 ℃ in the southern than in the northern populations. However, the fecundity of the first was higher than that of the second. According to the energy conservation law, we speculate that the shorter developmental period in the southern populations led to their advantages in fecundity. The overall adaptability of the southern populations was weak, and their fecundity was not as strong as that of the northern populations at 27 ℃. We speculated that because southern China is not a major wheat-producing area, the low genetic diversity of aphids in the region has led to poor overall adaptability in southern aphids. These findings are somewhat surprising given the fact that other research showed the hypothesis of "the hotter the better". This result may be explained by the variable migratory behaviors among aphids from different populations. Wheat aphids generally move northward with the southwesterly airflow in March, and then move southwardly with the northwesterly wind after August to become a source of insects on autumn seedlings in winter wheat areas in China. Therefore, the sources of aphids collected in the same place may be different, which also creates uncertainty in the results. The possible interference of migration cannot be ruled out. Therefore, further studies are required to evaluate the impact of aphid migration. *S. miscanthi* had an intermediate temperature preference (Turak et al., 2010). The negative effects of low and high temperatures to population parameters have been observed in other species (Castillo et al., 2006). Therefore, the influence of temperature on the control efficiency and population fitness of aphids has been estimated in many previous studies, such as of *Aphidius gifuensis* (McCalla et al., 2019). In future research, we will explore the impact of thermal extreme temperature on aphids and the population fitness on aphids.

In addition, geographical factors had a certain correlation with the reproductive ability of aphids at different temperatures. Aphids at cooler regions of high latitude display larger thermal tolerance ranges and occur below their thermal optima, climate warming could act to increase fitness, ultimately, would increase aphid populations and their potential as pests. The fecundity of *S. miscanthi* southern populations was generally higher than that from northern

locations. As microenvironments in which insects live has temperature heterogeneity (Sinoquet et al., 2007; Woods et al., 2015), aphids can alleviate the negative effects of climate change by generating mobile individuals in the microclimate as thermoregulation adaptation. *S. avenae* can take advantage of the spatial heterogeneity of the microenvironment in which it inhabits, and transfer itself to the moist soil surface through thermoregulation to avoid the adverse effects of high temperature (Kearney et al., 2009). The reproductive rates of populations from various locations declined with increasing of temperature, except that of the YC population, which may have been affected by its high altitude and unique geographical environment. Insects have a small thermal tolerance in low latitudes, but a large thermal tolerance at high latitudes (Huey et al., 1989). This finding is similar to the results of our research. Therefore, geographical conditions are potential factors affecting the growth and development of aphids. The trade-off is the negative correlation between these two life history traits, and the main pair is adult longevity and fecundity (Cingolani et al., 2019). Multiple mating increased reproduction in *Edessa meditabunda* (Hemiptera: Pentatomidae) and the cost of increasing fecundity was the reduction of longevity (Silva et al., 2012). In this study, there was no negative correlation between adult longevity and fecundity. Perhaps the trade-off between insect's flying ability and reproduction has not been taken into consideration (Guerra et al., 2007).

This study clarified the effects of different temperatures on the development and reproduction of different geographical populations of *S. miscanthi.* The results provide a theoretical basis for predicting potential aphid outbreak locations. Many insects adopt behavioral strategy to regulate their body temperature. Therefore, when we study the impact of temperature on population relationships, we will also consider the role of thermoregulatory behavior strategies in it. Assessing facultative endosymbionts in the different populations better reveal the aphid thermal tolerance (Burke et al., 2009; Ferrari et al., 2020). There are close links between aphid responses to heat and to infection. Under extremely harsh natural conditions, aphids will initiate diapause adaptation strategies (Denlinger et al., 2002). Further research on these questions would be a useful way of the effect of temperature on aphids.

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3

Chapter III: Analysis of genetic structure of *Sitobion miscanthi* (Takahashi) from six geographic populations in China based on mitochondrial and primary symbiotic gene

From **Sun, J**., Li, Q., Francis, F., Chen, J. 2021. Analysis of genetic structure of *Sitobion miscanthi* (Takahashi) from six geographic populations in China based on mitochondrial and primary symbiotic gene. *Communications in Agricultural and Applied Biological Sciences*.

3.1. Foreword

In the previous chapter, we studied the effects of different constant temperatures on life history traits of several population of *Sitobion miscanthi*. Based on our analysis of previous results, we decided to study the migration behavior of six aphid populations. The migratory behavior of small temperature-changing insects could be described well by genetic diversity. Here, aphid mitochondrial *COI* gene was combined with two primary symbiont genes from *B. aphidicola* for PCR amplification, sequencing and population genetic structure analysis. Genetic structure analysis based on mitochondria and symbiotic genes were almost the same, indicating that symbiotic genes have potential significance in studying the genetic structure and diversity of aphid host populations. We hypothesize that the difference in annual average temperature at the collection site may be the cause of the genetic structure of diversity. In summary, understanding the genetic structure of different geographic populations of *S. miscanthi* can provide theoretical significance for the migration of aphids and further effects for strategies to control this pest species in wheat crop.

3.2. Abstract

Sitobion miscanthi (Takahashi) is one of the most destructive wheat pests in China. It could cause severe damages to wheat relying on its strong flight ability through airflow to carry the rapid spread followed by strong reproduction. The current reports on the genetic structure of aphid populations are mainly based on their mitochondrial genes. The primary symbiont *Buchnera aphidicola* occurs in almost all aphids while its potential role in studying the genetic structural diversity of aphid populations is still unknown. Our study was based on 6 geographical populations of *S. miscanthi* through China (Wuhan, WH; Suzhou, SZ; Kunming, KM; Langfang, LF; Tai'an, TA; Yichuan, YC). The mitochondrial *COI* gene of the aphid was combined with two single copy genes of the primary *B. aphidicola* symbiont for PCR amplification, sequencing and analyzing to investigate aphid population genetic structure. Selected geographical populations of *S. miscanthi* were divided into three groups, Yinchuan, Suzhou, and other populations according to the three genetic marker genes (*COI*: F_{CT} = 0.3642, P <0.05; *gnd*: F_{CT} = 0.4033, P < 0.05; *trpA*: F_{CT} = 0.2229, P <0.05), with significant genetic differentiation among the three groups. The use of mitochondrial and symbiotic genes gace similar results, which indicates that the symbiotic gene has potential meaning in studying the genetic structure and diversity of the aphid population. In addition, we found that the annual average temperature in Yinchuan and Suzhou areas was the highest and lowest in the six regions, which were 18.5℃ and 11.5℃, respectively. Therefore, we hypothesized that the difference in the annual average temperature of the collection places may be the reason for the diversity genetic structure. In summary, understanding the genetic structure of different geographic population of *S. miscanthi* may provide theoretical meaning

for the aphid migration and further ideas to control this pest.

Keywords

Sitobion miscanthi (Takahashi), population genetic structure, mitochondrial genes, primary symbiotic genes, genetic differentiation

3.3. Introduction

Migratory behavior is exhibited by numerous animal species and is recognized as one of the most fascinating natural phenomena (Dodson, 1988; Nelson, 1998; Sword et al., 2005). Insects correspond to the most species-rich class in the animal kingdom. Throughout the year, billions of insects migrate across the globe (Zhan et al., 2014). Insect migration is a key process to maintain the population dynamics of many pests in a large area (Ghosh et al., 2019). Over the past few decades, understanding the migration of a few insects has provided insights into migration routes and the underlying physiology and ecology of migration, with impacts ranging from pest control to management. However, relative to the migration of mammals and birds, little is known about insects.

The wheat aphid, *Sitobion miscanthi* (Takahashi), is one of the most important pests in China. *S. miscanthi* is Asiatic in origin and is widespread in China, India and the Far East and in the Pacific region (Blackman et al., 2000). Wheat aphid outbreaks occur and cause heavy damage every year, and knowledge of its population genetics and associated factors is fundamentally crucial for species management and conservation strategies (Yang et al., 2017). China offers good conditions for studying the regional migration of wheat aphids because of its large size and range of climate types (Liu et al., 2019).

Up to now, a lot of studies have focused on the morphology and biological characteristics of *S. miscanthi* (David, 1975; Sekhar et al., 1999). However, the factors for the population structure of *S. miscanthi* in China remained unknown. In the present study, we investigated its genetic diversity, population structure and demographic history using sequences of mitochondrial and primary symbiotic data. The objectives of this study were to reveal the genetic distribution of *S. miscanthi* related to current factors geographical barriers and ecological factors.

3.4. Materials and Methods

3.4.1. Sample collection

A total of 316 adult *S. miscanthi* individuals were collected from 6 locations (Figure 3-1), covering southern and northern distributions in China. All specimens were stored in absolute ethanol at - 20 °C until DNA extraction.

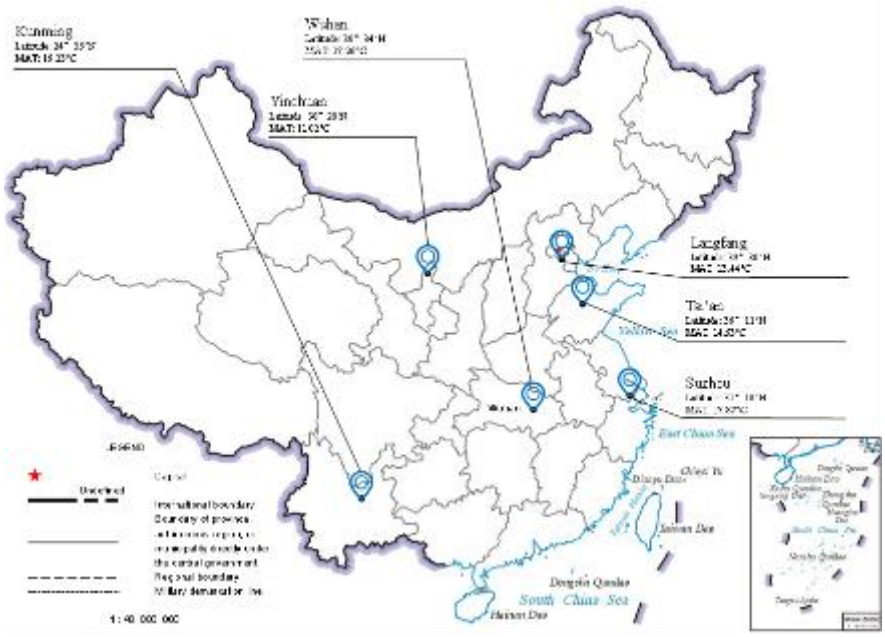


Figure 3-1. Map of China showing the locations of the sampling sites and the mean annual temperature (MAT) were calculated.

3.4.2. DNA extraction and sequencing

Genomic DNA was extracted from single adult aphids using a TIANamp genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China). Considering the genetic variability of different genes, one mitochondrial protein-coding gene (a partial sequence of *COI*) (Foottit et al., 2010) and two symbiotic genes (*gnd* and *trpA*) (Skurray et al., 1978; Wernegreen et al., 2000) were used as molecular markers. The PCR amplifications were performed using TaKaRa rTaq polymerase (TaKaRa Biomedical, Japan) in a total volume of 25 μL with the following conditions: an initial denaturation at 94°C for 0.5-1 min; followed by 35-40 cycles of 30 s at 94°C, 30 s at 51-60°C, and 1 min at 72°C; and a final extension step at 72°C for 10 min. The PCR products were visualized on 1.0% agarose gels under UV light. Purified PCR products were sequenced in both directions (Sanbo Biotechnology Co., Ltd., Beijing, China).

3.4.3. Population genetic diversity and structure

Sequences of mitochondrial and primary symbiotic markers were aligned independently using Clustal W implemented in MEGA version 5 with default parameters. Alignment of nucleotide sequences of the mitochondrial protein-

coding genes (*COI*, *gnd* and *trpA*) were inferred from the amino acid alignment. The number of polymorphic sites (S), the number of haplotypes (Ht), haplotype diversity (Hd), nucleotide diversity (π) and average number of nucleotide differences (K) for each location were calculated using Arlequin version 3.5.2.2. Several approaches were used in order to understand the population genetic structure of *S. miscanthi*. Paired F_{ST} analysis was also calculated using Arlequin to estimate the genetic differentiation between population pairs. It computes pairwise F_{ST} value for all pairs of populations as well as different indices of dissimilarities (genetic distances) between pairs of populations (Reynolds et al. 1983; Slatkin et al. 1995). Then, the network was rebuilt using NETWORK version 5 (Bandelt et al. 2000). A split network was constructed to reveal the relationships between the haplotypes.

3.4.4. Hierarchical analysis

For hierarchical analysis of molecular variance and to test group definition, a spatial analysis of molecular variance (SAMOVA) was performed using SAMOVA version 1.051 (Dupanloup et al. 2002). The number of groups ranged from 2 to 5, and the values of the fixation indices were compared among different group numbers with 1 000 permutations.

3.5. Results

3.5.1. Genetic diversity

Three different genes, *COI*, *gnd*, and *trpA*, in the 6 aphid populations showed genetic variation (Table 3-1). We found 33, 6 and 19 haplotypes of *COI*, *gnd*, and *trpA*, respectively, in 316 individuals. Yinchuan and Tai'an had their own unique haplotypes of each of the three molecular marker genes. *COI* contained the highest nucleotide diversity.

Gene	Number of samples	Length of sequences (bp)	Number of haplotypes	Number of mutation sites
COI	79	529	33	34
gnd	253	771	6	19
trpA	316	155	19	15

Table 3-1. Variation of sequences of different *Sitobion miscanthi* geographical populations

Further analysis showed that in all populations, the number of mutation sites in the *COI* sequence was 6-24 per population (Table 3-2), the number of haplotypes was 5-14, the haploid diversity was 0.45-0.98, the average number of nucleotide

differences was 1.87-6.62, and the nucleotide diversity was 0.0035-0.0125. The Wuhan population showed the highest number of mutation sites (24) and the highest haplotype diversity of 0.98. Wuhan also had the highest numbers of haplotypes (14). At the same time, the Wuhan and Yinchuan populations showed relatively high haplotype diversity (0.98 and 0.89), nucleotide diversity (0.0125 and 0.0081), and average nucleotide difference (6.62 and 4.26). By analyzing the genetic diversity parameters of the *COI* molecular markers, we could see obvious polymorphic differences among the aphid populations, and the genetic diversity of the Wuhan and Yinchuan populations was relatively high.

Table 3-2. Genetic diversity index of *Sitobion miscanthi* with *COI*, *gnd* and *trpA*[1)]

Population[2)]	S			Ht			Hd		
	COI	*gnd*	*trpA*	*COI*	*gnd*	*trpA*	*COI*	*gnd*	*trpA*
KM	—	17	6	—	3	6	—	0.417	0.37
WH	24	10	9	14	2	7	0.983	0.365	0.572
SZ	6	10	3	5	2	3	0.8	0.043	0.082
TA	11	12	6	9	3	7	0.449	0.441	0.464
YC	15	17	7	8	5	9	0.894	0.569	0.71
LF	—	10	2	—	2	2	—	0.313	0.394

Population[2)]	K			π		
	COI	*gnd*	*trpA*	*COI*	*gnd*	*trpA*
KM	—	4.984	0.666	—	0.00646	0.0043
WH	6.625	3.654	1.177	0.01252	0.00474	0.0076
SZ	2.267	0.426	0.125	0.00428	0.00055	0.00081
TA	1.865	4.265	0.869	0.00353	0.00553	0.0056
YC	4.258	5.01	1.556	0.00805	0.0065	0.01004
LF	—	3.134	0.788	—	0.00406	0.00509

[1)] S, number of variable sites; Ht, number of unique haplotypes; Hd, haplotype diversity; π, nucleotide diversity; K, average number of nucleotide differences.

[2)] KM, Kunming; WH, Wuhan; SZ, Suzhou; TA, Tai'an; YC, Yinchuan; LF, Langfang.

– indicates unable to obtain sequences.

The genetic diversity analysis of the *gnd* sequences showed that the number of sequence variation sites per population ranged from 10-17, and the number of haplotypes ranged from 2-5 (Table 3-2). Yinchuan showed the highest haplotype diversity (0.57), nucleotide diversity (0.0065), and average number of nucleotide differences (5.01). Kunming and Yinchuan showed high numbers of mutation sites (17). This molecular marker had a large number of variable sites but a small

number of haplotypes.

The genetic diversity analysis of *trpA* showed that the number of sequence variation sites per population ranged from 2-9 (Table 3-2), and the number of haplotypes ranged from 2-9. The haplotype diversity was 0.08-0.71, the nucleotide diversity was 0.0008-0.01, and the average number of nucleotide differences was 0.13-1.17. Wuhan showed the highest number of mutation sites (9), and Yinchuan contained the most haplotypes. The genetic diversities of the populations in Yinchuan and Wuhan were relatively high, and the genetic diversity of the populations in Suzhou was relatively low.

There were 33 haplotypes for the *COI* marker. Among these identified haplotypes, 28 were unique. Three (H2, H13, and H23) were the most widely distributed, accounting for 8.86, 39.24, and 6.33% of the total, respectively. In addition, we found 34 polymorphic sites, which consisted of 20 parsimony-informative sites and 14 singleton variable sites, representing 3.78 and 2.65% of the total, respectively. Across the 6 populations, the Hd was 0.835, and the nucleotide diversity (Pi) was 0.00845.

There were 6 haplotypes for *gnd*, two of which were unique. H1 and H2 were the most widely distributed, accounting for 68.77 and 26.09% of the total, respectively. In addition, we found 19 polymorphic sites, which consisted of 17 parsimony-informative sites and 2 singleton variable sites; their proportions were 2.21 and 0.26%, respectively. Across the 6 populations, the Hd for *gnd* was 0.459, and the Pi was 0.00622.

Finally, 19 haplotypes were observed for *trpA*. Among these identified haplotypes, 10 were unique. Two (H1 and H2) were the most widely distributed and accounted for 19.94 and 70.57% of the total, respectively. In addition, we found 15 polymorphic sites, which consisted of 8 parsimony-informative sites and 7 singleton variable sites, with proportions of 5.16 and 4.52%, respectively. Across the 6 populations, the Hd was 0.472, and the Pi was 0.00632.

3.5.2. Population genetic structure

The pairwise F_{ST} values for the different genes were very different. The pairwise F_{ST} is usually used to measure the degree of genetic differentiation between populations, and its value ranges between 0 and 1. $F_{ST}\leq 0.05$ indicates that there is almost no genetic differentiation between the two groups, $0.05<F_{ST}\leq 0.15$ indicates that the two populations have a moderate degree of genetic differentiation, $0.15<F_{ST}\leq 0.25$ indicates that the two populations have a high degree of genetic differentiation, and $F_{ST}>0.25$ indicates that the population is extremely differentiated.

The pairwise F_{ST} values based on *COI* were in range of 0.11579-0.91304 (Table 3-3). Most pairwise F_{ST} greater than 0.25 indicated that the populations

were extremely differentiated. However, the Wuhan population exhibited a moderate degree of genetic differentiation from the Langfang (0.11579). The Wuhan population exhibited a high degree of genetic differentiation from the Suzhou and Tai'an populations, respectively (0.24064 and 0.15221).

Table 3-3. Pairwise F_{ST} values of the *Sitobion miscanthi* populations based on the mitochondrial genes of *COI*

Population	SZ	WH	KM	TA	LF	YC
SZ	0					
WH	0.24064	0				
KM	0.85470	0.55274	0			
TA	0.53411	0.15221	0.85625	0		
LF	0.54667	0.11579	0.90354	0.41341	0	
YC	0.39416	0.21193	0.73933	0.41614	0.38084	0

The pairwise F_{ST} values based on *gnd* were in the range of 0.00075-0.70148 (Table 3-4). The Langfang population exhibited almost no genetic differentiation from the Wuhan, Kunming and Tai'an populations, respectively (0.02422, 0.01005 and 0.00075). The Tai'an population exhibited almost no genetic differentiation from the Wuhan and Kunming populations, respectively (0.01209 and 0.00340). Pairs of Wuhan with Kunming also exhibited almost no genetic differentiation (0.01076). Pairs of Suzhou with Langfang also exhibited a moderate degree of genetic differentiation (0.13961). The Suzhou population exhibited almost a high degree of genetic differentiation from the Wuhan, Kunming and Tai'an populations, respectively (0.16993, 0.17849 and 0.20663). The Yinchuan population exhibited a high degree of genetic differentiation from the other 5 populations Wuhan, Kunming, Tai'an, Langfang and Yinchuan populations, respectively (0.70148, 0.43682, 0.38106, 0.37663 and 0.47521).

Table 3-4. Pairwise F_{ST} values of the *Sitobion miscanthi* populations based on the mitochondrial genes of *gnd*

Population	SZ	WH	KM	TA	LF	YC
SZ	0					
WH	0.16993	0				
KM	0.17849	0.01076	0			
TA	0.20663	0.01209	0.00340	0		
LF	0.13961	0.02422	0.01005	0.00075	0	
YC	0.70148	0.43682	0.38106	0.37663	0.47521	0

The pairwise F_{ST} values based on *trpA* ranged from 0.01325-0.46479 (Table 3-5). Most pairwise F_{ST} less than 0.05 indicated that there was almost no genetic differentiation between populations. However, the Yinchuan population exhibited an extremely differentiated of genetic differentiation from the Suzhou and Kunming (0.46479 and 0.30475), and exhibited a high degree of genetic differentiation from the Wuhan, Tai'an and Langfang populations, respectively (0.15970, 0.21046 and 0.18028). Pairs of Suzhou with Langfang also exhibited a high degree of genetic differentiation (0.19097). Pairs of Suzhou with Wuhan and Kunming exhibited a moderate degree of genetic differentiation (0.13079 and 0.12019).

Table 3-5. Pairwise F_{ST} values of the *Sitobion miscanthi* populations based on the mitochondrial genes of *trpA*

Population	SZ	WH	KM	TA	LF	YC
SZ	0					
WH	0.13079	0				
KM	0.04318	0.01994	0			
TA	0.12019	0.01051	0.01464	0		
LF	0.19097	0.01421	0.03073	0.01325	0	
YC	0.46479	0.15970	0.30475	0.21046	0.18028	0

The SAMOVA results showed that the F_{ST}, F_{CT} and F_{SC} values decreased from 2 to 5. When K was 3, F_{ST} was lowest, and F_{CT} was highest. Although the groups did not yield the same partitioning, Suzhou and Yinchuan were different from the other four populations, respectively.

3.5.3. Haplotype phylogeny

Phylogenetic trees were constructed using the neighbor-joining method for the three molecular marker haploid sequences of *S. miscanthi*. The same species of aphid was selected as the outgroup for each tree, and their taxonomic relationship was relatively close.

Cluster analysis of the mitochondrial haplotype sequences showed that H18 (Wuhan) and H33 (Kunming) were significantly different from the other haplotypes (Fig. 3-2). There was no obvious differentiation among the other populations, and there was a certain degree of gene flow among the various populations.

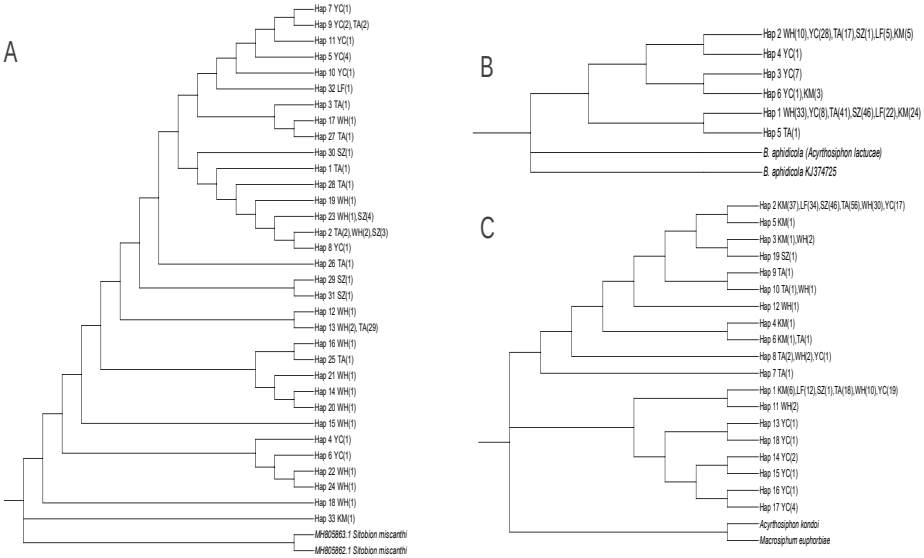


Figure 3-2. Neighbour-joining phylogenetic trees of the haplotypes of *Sitobion miscanthi* from China based on *COI* (A), *gnd* (B), and *trpA* (C). The numerical label beside each haplotype is the designated name of each haplotype.

Cluster analysis of the haplotype sequences of *gnd* showed that the tree was clearly divided into two large clusters, of which H1 and H5 made up one group, and the remaining haplotypes made up another group. Tai'an and Yinchuan had unique haplotypes.

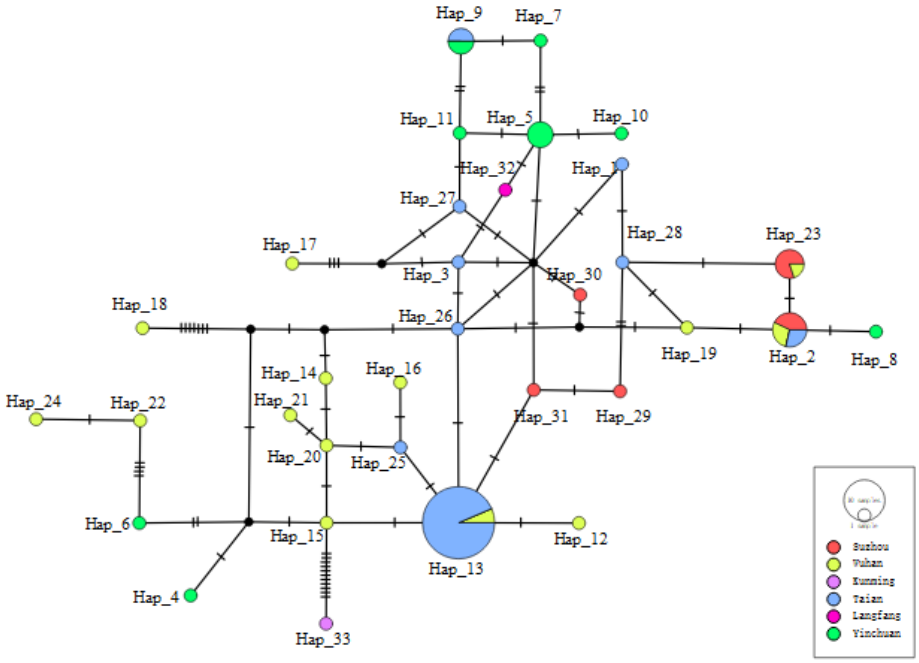
The *trpA* haplotype phylogenetic tree was also divided into two large clusters. Only Langfang didn't have its unique haplotypes.

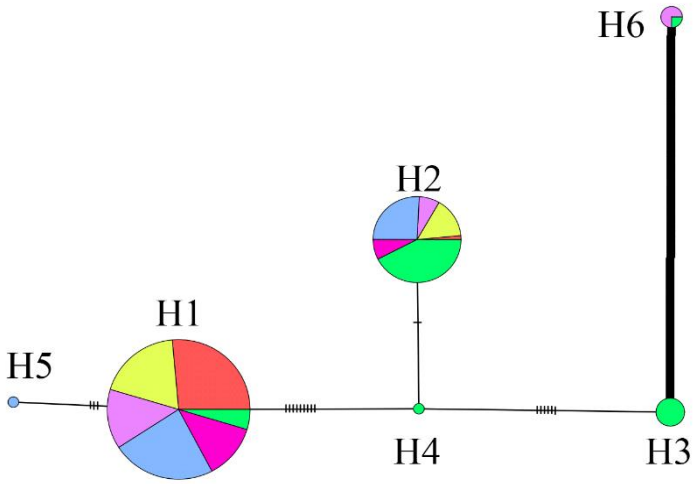
3.5.4. Haplotype network

In a haplotype network graph, the ancestral haplotype should generally be located in the middle of the network graph and be widely distributed in the geographical area; the most recent haplotype should be located at the edge of the network graph, and its geographical distribution range should be very limited. Here, the haplotype network maps had a radial distribution, that is, most haplotypes had only a single individual. Some high-frequency and widely

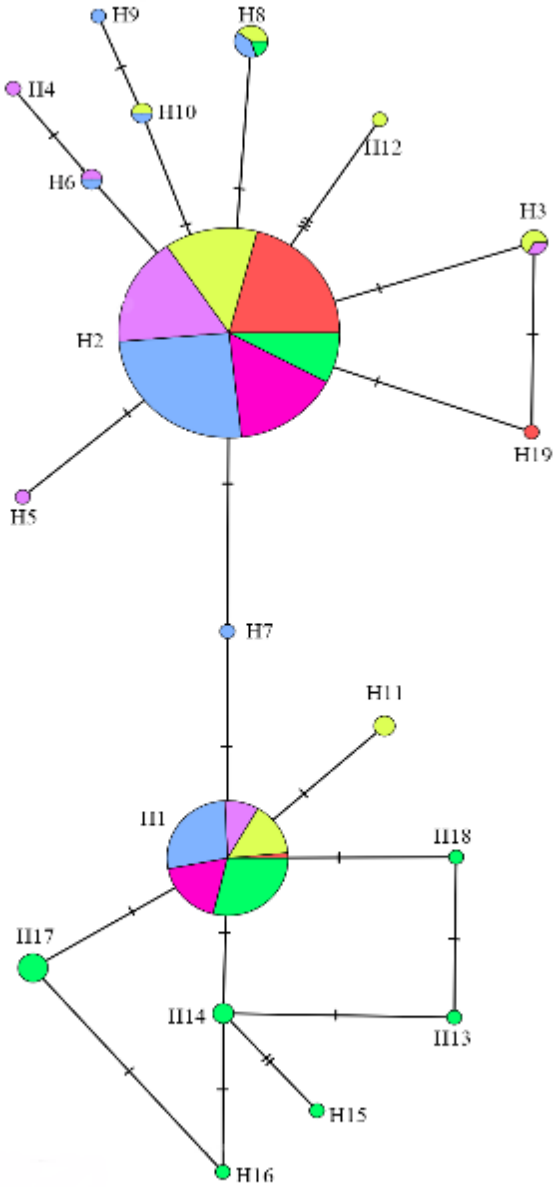
distributed haplotypes were located in the center of the network map, while haplotypes with narrower geographical distributions were located at the edge of the network graph. The network diagram of the mitochondrial gene *COI* showed that the 33 haplotypes were divided into two groups (Figure 3-3A). The high-frequency haplotypes were H2, H13 and H23, which represented the majority of the haplotypes identified, probably reflecting that these three haplotypes may be ancestral. The remaining rare haplotypes were located at the edges and along the connections of the network graph and were linked to the ancestral haplotypes through mutations. Most of the rare haplotypes were linked to an ancestral haplotype through one or more mutations, and a few were obtained by multiple successive mutations of the ancestral haplotype. Some haplotypes were missing, which may be due to insufficient sampling, which could result in the absence of the expected haplotypes, or due to evolution, during which some of the haplotypes may have disappeared from the extant populations.

(A)





(B)



(C)

Figure 3-3 Haplotype network of *Sitobion miscanthi* from China based on *COI* (A), *gnd* (B), and *trpA* (C). Different colours represent different sampling locations.

The *gnd* network graph (Figure 3-3B) was a large starshaped network centered on haplotypes H1 and H2.

The network map formed by the *trpA* gene haplotypes (Figure 3-3C) showed that H1 and H2 were in the middle of the network map and formed the centers of two large star-shaped subnetworks. The remaining rare haplotypes were connected to H1 or H2 by mutations and were located on the edges of the network graph.

3.5.5. Historical population dynamics

When analyzing the historical dynamics of a population, neutrality testing and mismatch analysis are usually used. Neutrality test analysis uses parameter values to determine whether a population is a stable population or a recently formed population that has undergone expansion. Theoretically, in a stable population, the values of Fu's Fs and Tajima's D are close to 0. Significant negative value indicates that the population has undergone a sudden increase. Significant positive value indicates that the population may have differentiated, leading to the formation of a subpopulation, or that the population has undergone a bottleneck event. In mismatch analysis, when a population is a long-lived and stable population, the mismatch distribution map is usually multimodal. When a population is a recently formed or expanded population, the mismatch distribution map is usually unimodal. When the historical dynamics of each population were analyzed separately, the Fu's Fs values showed significant negative values with *COI* and *trpA* genes, indicating that the populations had expanded.

3.6. Discussion

The active migration ability of *S. miscanthi* is limited, but it can spread by wind and other means. The traditional marker recovery method can be used to determine the migration and migration pathway of large insects (Chen et al., 2012). But the aphid is small and difficult to recover, so the traditional chemical dye labeling is difficult to achieve.

In recent years, the research on insect migration shows that molecular biology research is a powerful way to analyze species diffusion (He.et al, 2008). Yang et al. used *COI* gene and other markers to reveal that *Plutella xylostella* migrated from the middle and lower reaches of the Yangtze River to northern China and finally arrived in the northeast (Yang., 2015). Xu et al. studied *Sitobion avenae* of 17 geographical populations in China by using *COI* gene and other markers, and speculated that the southern insect sources with different haplotypes would migrate to the northern population in early spring (Xu et al., 2011). Prabhulinga et al. analyzed the genetic diversity and geographical structure of cotton whitefly in 22 regions of India by using mitochondrial *COI* gene, and confirmed that the

whitefly in India is isolated according to geographical distance (Prabhulinga et al., 2021). At the same time, the primary symbiont of aphids has a far-reaching impact on the distribution and evolution of hosts. Therefore, some studies have also used the primary symbiotic gene as a candidate gene marker. Zhang et al. used the *atp*AGD gene of aphid primary symbiont *B. aphidicola* to analyze the genetic diversity of 8 different geographical populations of the sumac gall aphid *Schlechtendalia chinensis* in China. The results showed that 16 haplotypes of *atp*AGD gene were divided into north-south evolutionary branches separated by the Yangtze River, suggesting that the contemporary genetic structure is likely to be affected by historical geological events (Zhang et al., 2018). Therefore, the primary symbiotic genes of aphids can be used to study the differences of genetic diversity among different populations of aphids.

Our 6 different geographical populations of *S. miscanthi* were divided into three groups, Yinchuan, Suzhou, and other populations according to the three genetic marker genes, and there was significant genetic differentiation among the three groups. Mitochondrial and symbiotic genes gave similar conclusions, which indicated that the symbiotic gene had potential meaning in studying the genetic structure and diversity of the aphid population. In addition, we found that the annual average temperature in Yinchuan and Suzhou areas was the highest and lowest in the six regions respectively. Therefore, we hypothesis that the difference in the annual average temperature of the collection places may be the reason for the diversity genetic structure. Understanding the genetic structure of different geographic population of *S. miscanthi* may provide theoretical meaning for the aphid migration. This study investigated the population genetics, demographic history and evolutionary adaptation of *S. miscanthi* in main wheat native range in China. We have rejected the hypothesis that this invasive pest had been introduced into Western China in the last couple of decades. An understanding of the level and pattern of genetic variation in native populations can provide valuable insights for speculating on the migration of this destructive pest species.

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4

Chapter IV: Population genetic structure of *Sitobion miscanthi* in China

From **Sun, J**., Li, Q., Tan, X., Fan, J., Zhang, Y., Qin, Y., Francis, F., Chen, J. 2020. Population genetic structure of *Sitobion miscanthi* in China. Journal of integrative agriculture, 19(0), 2-11.

4.1. Foreword

To better understand the migratory behavior of *S. miscanthi* and perform a more comprehensive and detailed study based on the results of the previous chapter, we then expanded the collection of samples and expanded the population locations. The places covered almost all major wheat producing areas in China. In this study, we analyzed 18 geographical populations in China by using one mitochondrial gene, the nuclear gene *EF-1α*, and two endosymbiotic genes *gnd* and *trpA* to study the population genetic structure and history of *S. miscanthi*. Data analysis of each population showed high haplotype diversity and low nucleotide diversity. SAMOVA analysis did not find a correlation between genetic and geographic distances. We inferred that these aphids appeared first in the southwest and south regions and spread to the north with the help of the southeast and southwest monsoons, which occur in spring and summer. In autumn, the aphids spread southward with the northeast and northwest monsoons. There are two main natural migration pathways of *S. miscanthi* in China. One is from Yunnan to the Sichuan Basin, and the other is from the Wuhan, Xinyang and Jiaodong Peninsula areas to the northwest.

4.2. Abstract

The wheat aphid, *Sitobion miscanthi*, is one of the most destructive pests of wheat plants in the temperate regions of China. Little is known about the genetic structure of this aphid and the effects of different locations on its population. In this study, we investigated the population genetic structure and demographic history of *S. miscanthi* by analysing 18 geographical populations across China using one mitochondrial gene; one nuclear gene, *EF-1α*; and two endosymbiont *Buchnera* genes, *gnd* and *trpA*. Data analysis of the various groups showed high haplotype diversity and low nucleotide variation. SAMOVA analysis did not find a correlation between genetic and geographic distances. However, areas with high population diversity exhibited high haplotype diversity. Therefore, we speculate that there are two main natural migration pathways of *S. miscanthi* in China. One is from Yunnan to the Sichuan Basin, and the other is from the Wuhan, Xinyang and Jiaodong Peninsula areas to the northwest. Based on this hypothesis, we inferred that these aphids appeared first in the southwest and south regions and spread to the north with the help of the southeast and southwest monsoons, which occur in spring and summer. In autumn, the aphids spread southward with the northeast and northwest monsoons.

Keywords

Sitobion miscanthi, molecular marker*, COI,* symbiotic bacteria, phylogenetic tree

4.3. Introduction

During evolution, many insects have developed seasonal migration behaviours (Dingle 1972; Danks 1978; Dingle 1982; Alerstam and Kesson 2003; Hasiotis *et al*. 2003; Christer *et al*. 2008). By this means, the population can reduce competition, avoid natural enemies and evade cold winter weather (Friedberg *et al*. 1993; Williams 2001; Watkinson *et al*. 2010). Understanding the migration behaviour of agricultural pests can lead to more effective control of these pests and prevent large outbreaks, which is of great significance to agricultural security (Hill 1975; Nair *et al*. 2000; Thomson *et al*. 2010). Fluorescence labelling and radioisotope methods are commonly used to study the migration of large animals (Skrisovska and Frédéric 2008). However, insects, such as aphids, are small and difficult to mark, so it is difficult to study their migration with traditional methods. Using population genetics has thus become the primary method of studying insect migration (Black *et al*. 2001; Ravel *et al*. 2001; Chapman *et al*. 2010; Lindroth 2012). In recent years, mitochondrial DNA *COI* has been approbated as the standard consensus sequences (Ratnasingham and Hebert 2007). Elongation factor (*EF-1α*) is one kind of single copy nuclear gene for encoding protein (Normark and Benjamin 1999), which plays an important role in higher taxa phylogeny. Some scholars have studied aphids by symbiotic bacteria of *Buchnera* combined with others genes. The common genes are the *gnd* gene (gluconate-6-phosphate dehydrogenase) and the *trpA* gene (Chen *et al*. 2013). Since the 1980s, the research on each biological species in these fields has mostly involved mitochondrial haplotyping *COI*, which is widely dispersed as a molecular marker for the study of genetic structure, biodiversity and molecular phylogeny and evolution. *EF-1α* gene was used as a standard molecular marker in the study of insect molecular phylogeny (Braby *et al*. 2006). The *gnd* gene combined with the *COI* gene were suggested by scientist to study aphid (Zhang *et al*. 2014).

The grain aphid, *Sitobion miscanthi*, is a destructive pest of wheat crops worldwide (Pietro *et al*. 1998; Winder *et al*. 1999; Larsson 2005; George *et al*. 2007; Xu *et al*. 2011). It causes severe yield loss by directly sucking plant sap and by transmitting viral diseases (Khan 2012). Biological, ecological and genetic studies have indicated that this aphid is migratory in many regions around the world. Although outbreaks of this pest occur annually in China and cause heavy damage, little is known concerning its migration within this region. To better understand its migration pattern, we investigated the population genetic structure and demographic history of *S. miscanthi* by analysing 18 geographical populations across China using one mitochondrial gene, one nuclear gene (*EF-1α*) and two symbiotic genes (*gnd* and *trpA*).

4.4. Materials and Methods

4.4.1. Sample collection

In this study, a total of 774 adult individuals of *S. miscanthi* were collected from 18 locations (Figure 4-1) representing the distribution of the species in China (Deng et al. 2016). All specimens were stored in absolute ethanol at -20°C until DNA extraction.

Figure 4-1. Topographical map of China with the sample localities represented by black dots.

4.4.2. DNA extraction and sequencing

Genomic DNA was extracted from single adult aphids using a TIANamp genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China). Considering the genetic variability of different genes, one mitochondrial protein-coding gene (a partial sequence of *COI*) (Foottit et al. 2010), one nuclear gene (*EF-1α*) (Penny et al. 2002) and two symbiotic genes (*gnd* and *trpA*) (Skurray et al. 2010; Wernegreen et al. 2010) were used as molecular markers. The PCR amplifications were performed using TaKaRa rTaq polymerase (Takara Biomedical, Japan) in a total volume of 25 μl with the following conditions: an initial denaturation at 94°C for 0.5-1 min; followed by 35-40 cycles of 30 s at 94°C, 30 s at 51-60°C, and 1 min at 72°C; and a final extension step at 72°C for 10 min. The PCR products were visualized on 1.0% agarose gels under UV light. Purified PCR products were

sequenced in both directions (Sanbo Biotechnology Co., Ltd., Beijing, China).

4.4.3. Population genetic diversity

Each sequence was independently aligned using Clustal W in MEGA version 10.1.1 (Kumar *et al*. 1994) with the default parameters. The nucleotide sequences of the coding genes (*COI*, *EF-1α*, *gnd* and *trpA*) were aligned according to their amino acid sequences (Abadi *et al*. 2019). Arlequin version 3.5.2.2 (Excoffier *et al*. 2010) was used to calculate the number of polymorphic sites (S), number of haplotypes (Ht), haplotype diversity (Hd), nucleotide diversity (π) and average number of nucleotide differences at each position (K). Paired F_{ST} analysis was also calculated using Arlequin to estimate the genetic differentiation between population pairs. It computes pairwise F_{ST}'s for all pairs of populations as well as different indices of dissimilarities (genetic distances) between pairs of populations. (Reynolds *et al*. 1983; Slatkin *et al*. 1995). Then, the network was rebuilt using NETWORK version 5 (Bandelt *et al*. 2000). A split network was constructed to reveal the relationships between the haplotypes.

4.4.4. Hierarchical analysis

For hierarchical analysis of molecular variance and to test group definition, a spatial analysis of molecular variance (SAMOVA) was performed using SAMOVA version 1.051 (Dupanloup et al. 2002). The number of groups ranged from 2 to 9, and the values of the fixation indices were compared among different group numbers with 1,000 permutations.

4.5. Results

4.5.1. Genetic diversity

Four different genes, *COI*, *EF-1α*, *gnd*, and *trpA*, in the 18 aphid populations showed genetic variation (Table 4-1). We found 55, 28, 9 and 47 haplotypes of *COI*, *EF-1α*, *gnd*, and *trpA*, respectively, in 774 individuals. Yinchuan and Qingdao had their own unique haplotypes of each of the four molecular marker genes. *COI* contained the highest nucleotide diversity.

Table 4-1. Variation of sequences of different *Sitobion miscanthi* geographical populations

Gene	Number of samples	Length of sequences (bp)	Number of haplotypes	Number of mutation sites
COI	224	529	55	34
EF-1α	696	457	21	47
gnd	517	771	9	23
trpA	774	155	47	27

Further analysis showed that in all populations, the number of mutation sites in the *COI* sequence was 2-24 per population (Table 4-2), the number of haplotypes was 2-17, the haploid diversity was 0.449-1, the nucleotide diversity was 1-6.625, and the average number of nucleotide differences was 0.00189-0.01252. The Wuhan population showed the highest number of mutation sites (24) and a high haplotype diversity of 0.983. Jinan showed a nucleotide difference of 0.00416 and had the highest haplotype diversity (1.000). Hefei, Wuhan and Fengyu had the highest numbers of haplotypes at 17, 14 and 9, respectively. At the same time, these three populations showed relatively high haplotype diversity (0.968, 0.983 and 0.729), nucleotide diversity (3.822, 6.625 and 1.527), and average nucleotide difference (0.00732, 0.01252, and 0.00289). In Xinxiang, only two haplotypes were found in the Pingliang and Yantai populations, making them the populations with the lowest number of haplotypes and indicating very low population genetic diversity. By analysing the genetic diversity parameters of the *COI* molecular markers, we could see obvious polymorphic differences among the aphid populations, and the genetic diversity of the Wuhan and Xinxiang populations was relatively high.

Table 4-2. Genetic diversity index of *Sitobion miscanthi* with *COI*

Population	S	Ht	Hd	K	π
FY	5	9	0.729	1.527	0.00289
MY	3	4	0.778	1.444	0.00273
WH	24	14	0.983	6.625	0.01252
SZ	6	5	0.8	2.267	0.00428
XZ	6	6	0.439	1.386	0.00262
HF	10	17	0.968	3.822	0.00732
QD	2	3	0.833	1	0.00189
TA	11	9	0.449	1.865	0.00353
JN	5	6	1	2.2	0.00416
YT	8	2	0.525	4.2	0.00794
PL	8	2	0.5	4	0.00765
YC	15	8	0.894	4.258	0.00805
XX	8	2	0.556	4.444	0.0084
XY	3	5	0.786	1.393	0.00263

S: Number of variable sites; Ht: Number of unique haplotypes; Hd: Haplotype diversity; π: Nucleotide diversity; K: Average number of nucleotide differences

Analysis of the *EF-1α* molecular markers showed that the number of sequence mutations per population ranged from 1 to 39, and the number of haplotypes ranged from 2 to 10 (Table 4-3). Yinchuan showed the highest haplotype diversity

(0.681), nucleotide diversity (0.00263), and average number of nucleotide differences (1.198). Tai'an showed a high number of mutation sites (39). The Mianyang, Suzhou, Qingdao and Yantai populations showed relatively low haplotype diversity (0.087, 0.042, 0.042 and 0.080), nucleotide diversity (0.0710, 0.00037, 0.00009 and 0.00018), and mutation site number (37, 4, 1 and 1). *EF-1α* showed relatively low population genetic diversity in the Jiaodong Peninsula and relatively high population genetic diversity in the North China Plain and Sichuan Basin populations.

Table 4-3. Genetic diversity index of *Sitobion miscanthi* with *EF-1α*

Population	S	Ht	Hd	K	π
KM	1	2	0.401	0.401	0.00088
FY	3	4	0.348	0.376	0.00083
MY	37	2	0.087	3.229	0.00710
WH	2	3	0.159	0.162	0.00036
SZ	4	2	0.042	0.167	0.00037
XZ	1	2	0.123	0.123	0.00027
LYG	37	4	0.131	1.738	0.00382
QD	1	2	0.042	0.042	0.00009
TA	39	6	0.208	1.493	0.00371
YT	1	2	0.080	0.080	0.00018
PL	3	4	0.357	0.418	0.00092
YC	11	10	0.681	1.198	0.00263
LF	4	5	0.137	0.168	0.00037
XX	37	3	0.099	1.908	0.00419
XY	2	3	0.590	0.716	0.00157

S: Number of variable sites; Ht: Number of unique haplotypes; Hd: Haplotype diversity; π: Nucleotide diversity; K: Average number of nucleotide differences

The genetic diversity analysis of the *gnd* sequences showed that the number of sequence variation sites per population ranged from 10-17, and the number of haplotypes ranged from 2-5 (Table 4-4). Qingdao showed the highest haplotype diversity (0.725), nucleotide diversity (5.879), and average number of nucleotide differences (0.00763). Kunming, Fengyu, and Yinchuan showed high numbers of mutation sites (17). This molecular marker had a large number of variable sites but a small number of haplotypes. Overall, *gnd* showed relatively low population genetic diversity in Jiangsu Province and relatively high population genetic diversity in the Jiaodong Peninsula and Northwest populations.

Table 4-4. Genetic diversity index of *Sitobion miscanthi* with *gnd*

Population	S	Ht	Hd	K	π
KM	17	3	0.417	4.984	0.00646
FY	17	3	0.345	3.588	0.00465
WH	10	2	0.365	3.654	0.00474
SZ	10	2	0.043	0.426	0.00055
XZ	10	2	0.111	1.111	0.00144
LYG	10	2	0.312	3.117	0.00404
HF	10	2	0.505	5.051	0.00655
QD	15	5	0.725	5.879	0.00763
TA	12	3	0.441	4.265	0.00553
JN	10	2	0.428	4.278	0.00555
YT	10	2	0.536	5.357	0.00695
YC	17	5	0.569	5.01	0.0065
LF	10	2	0.313	3.134	0.00406
XX	10	2	0.425	4.248	0.00551
XY	16	3	0.345	3.055	0.00396

S: Number of variable sites; Ht: Number of unique haplotypes; Hd: Haplotype diversity; π: Nucleotide diversity; K: Average number of nucleotide differences

The genetic diversity analysis of *trpA* showed that the number of sequence variation sites per population ranged from 2-10 (Table 4-5), and the number of haplotypes ranged from 2-12. The haplotype diversity was 0.082-0.789, the nucleotide diversity was 0.125-1.884, and the average number of nucleotide differences was 0.00081-0.01216. Hefei showed the highest number of mutation sites, and Pingliang contained the most haplotypes. The genetic diversity of the populations in the Jiaodong Peninsula and the northwest region was relatively high, and the genetic diversity of the populations in the southwest and Jiangsu regions was relatively low.

There were 55 haplotypes for the *COI* marker. Among these identified haplotypes, 11 were unique. Three (H1, H14, and H22) were the most widely distributed, accounting for 13.39, 7.59, and 33.04% of the total, respectively. In addition, we found 34 polymorphic sites, which consisted of 20 parsimony-informative sites and 14 singleton variable sites, representing 3.78 and 2.65% of the total, respectively. Across the 18 populations, the Hd was 0.864, and the nucleotide diversity (Pi) was 0.00821.

Table 4-5. Genetic diversity index of *Sitobion miscanthi* with *trpA*

Population	S	Ht	Hd	K	π
KM	6	6	0.37	0.666	0.0043
FY	9	10	0.416	0.759	0.0049

MY	4	5	0.3	0.533	0.00344
WH	9	7	0.572	1.177	0.0076
SZ	3	3	0.082	0.125	0.00081
XZ	6	6	0.249	0.462	0.00298
LYG	7	7	0.49	1.411	0.0091
HF	10	7	0.637	1.506	0.00972
QD	8	10	0.653	1.822	0.01176
TA	6	7	0.464	0.869	0.0056
JN	6	5	0.527	1.102	0.00711
YT	7	12	0.789	1.884	0.01216
PL	9	11	0.707	1.843	0.01189
YC	7	9	0.71	1.556	0.01004
LF	2	2	0.394	0.788	0.00509
TG	6	7	0.692	1.457	0.0094
XX	6	5	0.558	1.332	0.00859
XY	2	3	0.435	0.569	0.00367

S: Number of variable sites; Ht: Number of unique haplotypes; Hd: Haplotype diversity; π: Nucleotide diversity; K: Average number of nucleotide differences

Twenty-one haplotypes were found for *EF-1α*. H1, H3, H5, H11 and H16 were the five most widely distributed haplotypes and accounted for 12.13, 76.60, 1.71, 3.28, and 1.57% of the total, respectively. In addition, we found 47 polymorphic sites, which consisted of 39 parsimony-informative sites and 8 singleton variable sites, representing 8.53 and 1.75% of the total, respectively. The Hd across all 18 populations was 0.397, and the Pi was 0.00244.

There were 9 haplotypes for *gnd*, four of which were unique. H1, H2, and H8 were the most widely distributed, accounting for 66.54, 28.05, and 2.71% of the total, respectively. In addition, we found 23 polymorphic sites, which consisted of 19 parsimony-informative sites and 4 singleton variable sites; their proportions were 2.46 and 0.52%, respectively. Across the 18 populations, the Hd for *gnd* was 0.479, and the Pi was 0.00644.

Finally, 47 haplotypes were observed for *trpA*. Among these identified haplotypes, 26 were unique. Three (H1, H2, and H5) were the most widely distributed and accounted for 63.95, 20.28, and 2.33% of the total, respectively. In addition, we found 27 polymorphic sites, which consisted of 13 parsimony-informative sites and 14 singleton variable sites, with proportions of 8.39 and 9.03, respectively. Across the 18 populations, the Hd was 0.549, and the Pi was 0.00807.

4.5.2. Population genetic structure

The pairwise F_{ST} values for the different genes were very different. The pairwise F_{ST} is usually used to measure the degree of genetic differentiation between populations, and its value ranges between 0 and 1. $F_{ST}\leq0.05$ indicates that there is almost no genetic differentiation between the two groups, $0.05<F_{ST}\leq0.15$ indicates that the two populations have a moderate degree of genetic differentiation, $0.15<F_{ST}\leq0.25$ indicates that the two populations have a high degree of genetic differentiation, and $F_{ST}>0.25$ indicates that the population is extremely differentiated.

Based on *COI*, the pairwise F_{ST} values were in range of 0.00072-0.91304 (Table 4-6). Most pairwise F_{ST} greater than 0.25 indicated that the populations were extremely differentiated. However, the Wuhan population exhibited almost no genetic differentiation from the Pingliang, Taigu, Jinan and Qingdao populations, respectively (0.02757, 0.03636, 0.03492 and 0.00648). The Langfang population exhibited almost no genetic differentiation from the Pingliang and Yinchuan populations, respectively (0.00102 and 0.004267). Pairs of Fengyu with Taian, Mianyang with Suzhou, and Hefei with Lianyungang also exhibited almost no genetic differentiation, respectively (0.00072, 0.02073 and 0.00103).

Table 4-6. Pairwise F_{ST} values of the *Sitobion miscanthi* populations based on the mitochondrial genes of *COI*

Population	FY	HF	JN	KM	LF	LYG	MY	PL	QD	SZ	TA	TG	WH	XX	XY	XZ	YC	YT
FY	0																	
HF	0.20198	0																
JN	0.21944	0.19957	0															
KM	0.88127	0.74593	0.824	0														
LF	0.50918	0.12704	0.30526	0.6431	0													
LYG	0.64986	0.00103	0.62286	0.7958	0.4852	0												
MY	0.66506	0.29089	0.69923	0.91034	0.74	0.31579	0											
PL	0.27202	0.147	0.0677	0.69231	0.00102	0.36	0.63817	0										
QD	0.40136	0.34028	0.16418	0.91304	0.75	0.84	0.78679	0.05263	0									
SZ	0.56319	0.18102	0.58003	0.8547	0.54667	0.13333	0.02073	0.51417	0.67026	0								
TA	0.00072	0.1828	0.15711	0.85625	0.41341	0.60035	0.62799	0.18757	0.34035	0.53411	0							
TG	0.71517	0.11203	0.67805	0.6253	0.3519	0.4718	0.3	0.44828	0.86207	0.41667	0.66481	0						
WH	0.14735	0.11615	0.03492	0.55274	0.11579	0.06	0.31682	0.02757	0.00648	0.24064	0.15221	0.03636	0					
XX	0.59809	0.15886	0.49023	0.75155	0.13043	0.21212	0.25932	0.36064	0.56756	0.1848	0.558	0.25	0.27785	0				
XY	0.79744	0.69164	0.74017	0.90336	0.83571	0.8631	0.87066	0.69443	0.76168	0.82558	0.77304	0.87363	0.43019	0.76792	0			
XZ	0.43233	0.4239	0.49376	0.89866	0.69195	0.75119	0.74305	0.51606	0.60125	0.67372	0.38482	0.77693	0.32408	0.67537	0.79359	0		
YC	0.56677	0.2765	0.40747	0.75437	0.04267	0.33648	0.55779	0.31021	0.50146	0.48667	0.5303	0.41275	0.30345	0.30071	0.68136	0.50934	0	
YT	0.63865	0.31742	0.55009	0.77748	0.31429	0.09189	0.38607	0.46237	0.60692	0.33859	0.61104	0.06667	0.38281	0.1279	0.74088	0.58466	0.19157	0

The pairwise F_{ST} values based on *EF-1α* ranged from 0.00046-0.73003 (Table 4-7). The pairwise F_{ST} based on *EF-1α* showed little overall differentiation, but Fengyu, Kunming, and Xinyang were very different from the other populations. These three populations were extremely differentiated. Contrary to them, Mianyang and Fengyu indicated almost no genetic differentiation from others, respectively.

Table 4-7. Pairwise F_{ST} values of the *Sitobion miscanthi* populations based on the mitochondrial genes of *EF-1α*

Population	FY	HF	KM	LF	LYG	MY	PL	QD	SZ	TA	WH	XX	XY	XZ	YC	YT
FY	0															
HF	0.45681	0														
KM	0.00611	0.21514	0													
LF	0.64167	0.24118	0.58692	0												
LYG	0.29154	0.03159	0.15107	0.01932	0											
MY	0.34441	0.09243	0.19332	0.0439	0.00101	0										
PL	0.62063	0.1509	0.53322	0.07609	0.01928	0.03841	0									
QD	0.73003	0.21923	0.8041	0.01805	0.01462	0.03633	0.1321	0								
SZ	0.67467	0.20183	0.6592	0.01402	0.01394	0.03469	0.08911	0.01075	0							
TA	0.41363	0.13126	0.26771	0.01337	0.00046	0.00229	0.02398	0.01112	0.00794	0						
WH	0.24767	0.02105	0.12545	0.06386	0.00858	0.04448	0.0474	0.05402	0.05229	0.04515	0					
XX	0.29009	0.02314	0.14503	0.02401	0.01188	0.00024	0.02094	0.01789	0.01715	0.00281	0.00592	0				
XY	0.64589	0.1478	0.40897	0.59051	0.39202	0.43868	0.45368	0.52768	0.52181	0.502	0.3064	0.36866	0			
XZ	0.69554	0.20696	0.71223	0.02818	0.01542	0.03639	0.11101	0.05572	0.02741	0.01377	0.05331	0.01843	0.52045	0		
YC	0.52203	0.17618	0.38316	0.1841	0.06261	0.08584	0.04829	0.20072	0.17553	0.08734	0.07862	0.06346	0.49359	0.18911	0	
YT	0.68813	0.20704	0.68985	0.01181	0.01211	0.03446	0.09961	0.03105	0.01535	0.00494	0.05188	0.0168	0.52638	0.04167	0.17977	0

The pairwise F_{ST} values based on *gnd* were in the range of 0.00098-0.93856 (Table 4-8). Most populations were extremely differentiated. The Taian population exhibited almost no genetic differentiation from the Fengyu, Hefei, Kunming, Langfang and Lianyungang populations, respectively (0.00344, 0.03356, 0.00098, 0.0032 and 0.00033). The Lianyungang population exhibited almost no genetic differentiation from the Fengyu, Kunming and Langfang populations, respectively (0.02817, 0.01395 and 0.04298). Pairs of Fengyu with Kunming, Fengyu with Langfang, Langfang with Kunming, Qingdao with Hefei, Qingdao and Jinan, Suzhou with Mianyang and Xinxiang and Wuhan also exhibited almost no genetic differentiation, respectively (0.00896, 0.02428, 0.01005, 0.03936, 0.02881, 0.02104 and 0.03526).

Table 4-8. Pairwise F_{ST} values of the *Sitobion miscanthi* populations based on the mitochondrial genes of *gnd*

Population	FY	HF	JN	KM	LF	LYG	MY	PL	QD	SZ	TA	TG	WH	XX	XY	XZ	YC	YT
FY	0																	
HF	0.10122	0																
JN	0.38283	0.10635	0															
KM	0.00896	0.06524	0.31188	0														
LF	0.02428	0.11134	0.4066	0.01005	0													
LYG	0.02817	0.10874	0.40487	0.01395	0.04298	0												
MY	0.11335	0.34378	0.64229	0.14473	0.1273	0.13367	0											
PL	0.62192	0.13636	0.39394	0.62739	0.69231	0.71429	0.1158	0										
QD	0.30774	0.03936	0.02881	0.22045	0.32408	0.31825	0.62927	0.15147	0									
SZ	0.12279	0.39201	0.69493	0.17849	0.13961	0.1451	0.02104	0.2873	0.70316	0								
TA	0.00344	0.03356	0.2726	0.00098	0.0032	0.00033	0.17936	0.46762	0.19158	0.20213	0							
TG	0.26526	0.36984	0.61038	0.23531	0.30433	0.31258	0.3885	0.6208	0.5424	0.72118	0.24101	0						
WH	0.20593	0.23602	0.44396	0.18459	0.20995	0.20685	0.36538	0.0989	0.37906	0.4163	0.14952	0.07925	0					
XX	0.19725	0.18422	0.38319	0.15678	0.20474	0.20059	0.45364	0.12457	0.29612	0.52368	0.12261	0.1278	0.03526	0				
XY	0.752	0.64498	0.64506	0.6702	0.78226	0.78355	0.92999	0.80235	0.61303	0.93856	0.69483	0.87059	0.73063	0.7031	0			
XZ	0.26938	0.37372	0.61405	0.25787	0.31229	0.32059	0.65658	0.28571	0.57586	0.61814	0.24231	0.06567	0.06352	0.11405	0.86814	0		
YC	0.52931	0.32363	0.20091	0.45524	0.5382	0.53484	0.69106	0.51838	0.2	0.73818	0.4321	0.59766	0.44071	0.37264	0.48743	0.5983	0	
YT	0.39507	0.15738	0.1389	0.29871	0.42291	0.41891	0.76821	0.26108	0.08218	0.80916	0.25708	0.5496	0.243	0.1478	0.61198	0.56329	0.02708	0

The pairwise F_{ST} values based on *trpA* ranged from 0.00683-0.64256 (Table 4-9). Yinchuan and Jinan indicated almost no genetic differentiation from other populations, respectively.

Table 4-9. Pairwise F_{ST} values of the *Sitobion miscanthi* populations based on the mitochondrial genes of *trpA*

Population	FY	HF	JN	KM	LF	LYG	MY	PL	QD	SZ	TA	TG	WH	XX	XY	XZ	YC	YT
FY	0																	
HF	0.13924	0																
JN	0.44015	0.10696	0															
KM	0.01265	0.13346	0.44757	0														
LF	0.02875	0.0343	0.30567	0.03073	0													
LYG	0.07179	0.16291	0.4039	0.07967	0.11208	0												
MY	0.04776	0.23469	0.54281	0.06162	0.16524	0.02704	0											
PL	0.10688	0.00683	0.12685	0.10355	0.02852	0.0877	0.17113	0										
QD	0.11594	0.02057	0.13984	0.11739	0.04334	0.08054	0.16094	0.0078	0									
SZ	0.03728	0.28553	0.64256	0.04318	0.19097	0.13749	0.06172	0.23837	0.24443	0								
TA	0.01313	0.05926	0.33171	0.01464	0.01325	0.09918	0.11808	0.045	0.05807	0.12019	0							
TG	0.13105	0.18265	0.3968	0.14887	0.15786	0.01934	0.08361	0.11513	0.07451	0.24172	0.14249	0						
WH	0.01866	0.03136	0.26967	0.01994	0.01421	0.07748	0.10847	0.02113	0.03133	0.13079	0.01051	0.11041	0					
XX	0.03036	0.01348	0.23869	0.03603	0.01868	0.05981	0.12418	0.00247	0.00295	0.21472	0.01333	0.0812	0.02095	0				
XY	0.0935	0.25417	0.54585	0.12519	0.20818	0.03308	0.01269	0.1868	0.16108	0.20403	0.15616	0.02324	0.13555	0.14849	0			
XZ	0.0107	0.20311	0.53381	0.01198	0.10789	0.05868	0.01801	0.15905	0.16533	0.00812	0.06969	0.14059	0.07146	0.10189	0.08555	0		
YC	0.30914	0.05175	0.00527	0.30475	0.18028	0.27772	0.38138	0.05115	0.05745	0.46479	0.21046	0.26171	0.1597	0.12151	0.3797	0.37434	0	
YT	0.2102	0.02766	0.06924	0.20288	0.10918	0.18297	0.2664	0.02286	0.01984	0.34198	0.13596	0.16814	0.08465	0.05916	0.26072	0.26161	0.02069	0

The SAMOVA results showed that the F_{ST}, F_{CT} and F_{SC} values decreased from 2 to 5. When K was 3, F_{ST} was lowest, and F_{CT} was highest. However, the groups did not yield the same partitioning. There was no evidence that *S. miscanthi* had a direct, close relationship between genetic and geographic distances.

4.5.3. Haplotype phylogeny

Phylogenetic trees were constructed using the neighbour-joining method for the four molecular marker haploid sequences of *S. miscanthi*. The same species of aphid was selected as the outgroup for each tree, and their taxonomic relationship was relatively close.

Cluster analysis of the mitochondrial haplotype sequences showed that H31 (Qingdao) and H44 (Xinyang) were significantly different from the other haplotypes (Figure 4-2). There was no obvious differentiation among the other populations, and there was a certain degree of gene flow among the various populations.

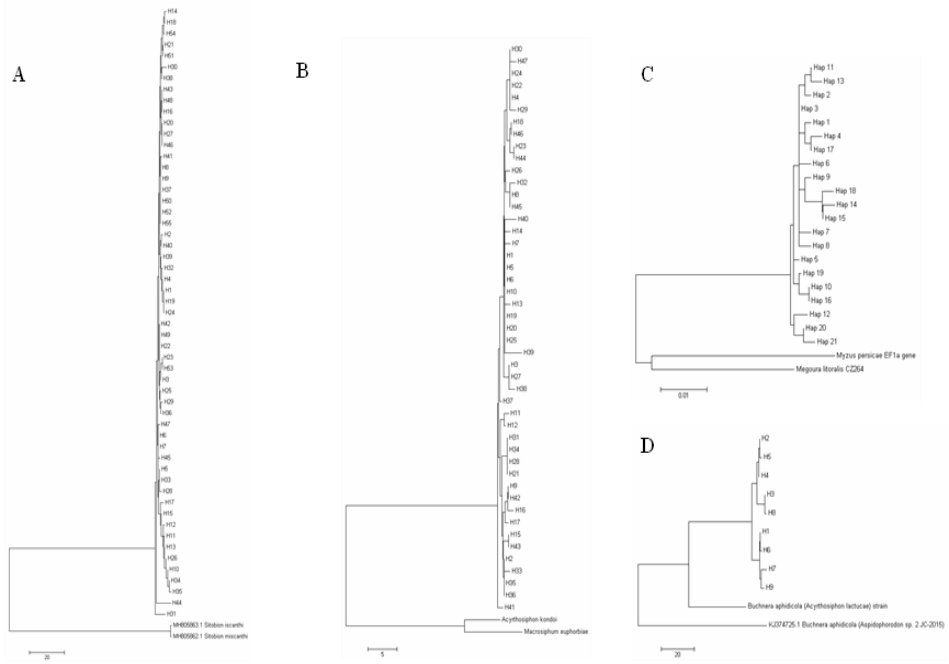


Figure 4-2. Neighbour-joining phylogenetic trees of the haplotypes of *Sitobion miscanthi* from China based on *COI* (A), *trpA* (B)*, EF-1α* (C), and *gnd* (D). ‘‘Hap’’ with a number was used as the haplotype name.

Cluster analysis of the *EF-1α* haplotype sequences showed that the haplotypes H12, H20 and H21 from Pingliang and Yinchuan were significantly different from the other haplotypes. There was not much differentiation among the other populations. Cluster analysis of the haplotype sequences of *gnd* showed that the tree was clearly divided into two large clusters, of which H1, H6, H7, and H9 made up one group, and the remaining haplotypes made up another group. Qingdao, Taian and Yinchuan had unique haplotypes.The *trpA* haplotype phylogenetic tree was also divided into two large clusters.

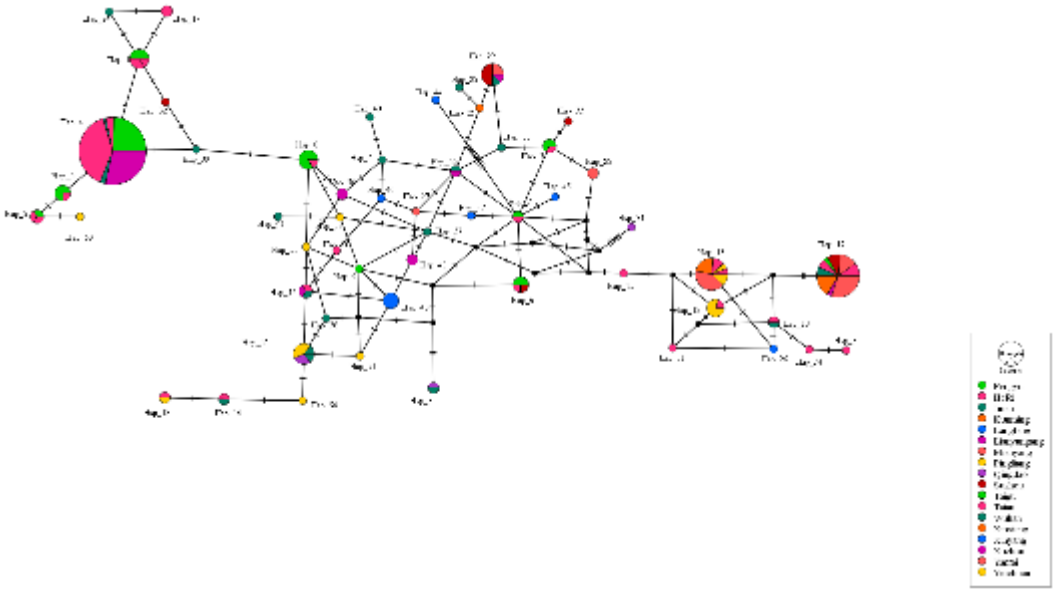
4.5.4. Haplotype network

In a haplotype network graph, the ancestral haplotype should generally be located in the middle of the network graph and be widely distributed in the geographical area; the most recent haplotype should be located at the edge of the network graph, and its geographical distribution range should be very limited. Here, the haplotype network maps had a radial distribution, that is, most haplotypes had only a single individual. Some high-frequency and widely distributed haplotypes were located in the center of the network map, while haplotypes with narrower geographical distributions were located at the edge of

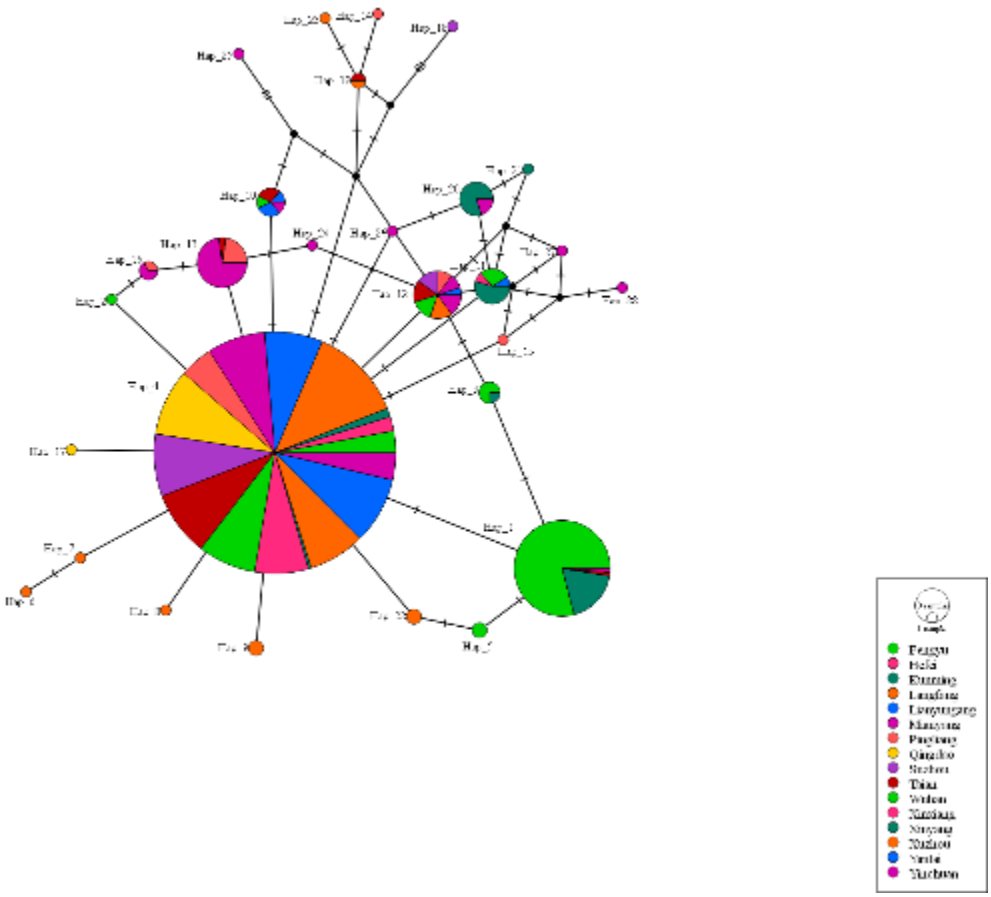
the network graph.

The network diagram of the mitochondrial gene *COI* showed that the 55 haplotypes were divided into two groups (Figure 4-3A). The high-frequency haplotypes were H4, H13 and H17, which represented the majority of the haplotypes identified, probably reflecting that these three haplotypes may be ancestral. The remaining rare haplotypes were located at the edges and along the connections of the network graph and were linked to the ancestral haplotypes through mutations. Most of the rare haplotypes were linked to an ancestral haplotype through one or more mutations, and a few were obtained by multiple successive mutations of the ancestral haplotype. Some haplotypes were missing, which may be due to insufficient sampling, which could result in the absence of the expected haplotypes, or due to evolution, during which some of the haplotypes may have disappeared from the extant populations.

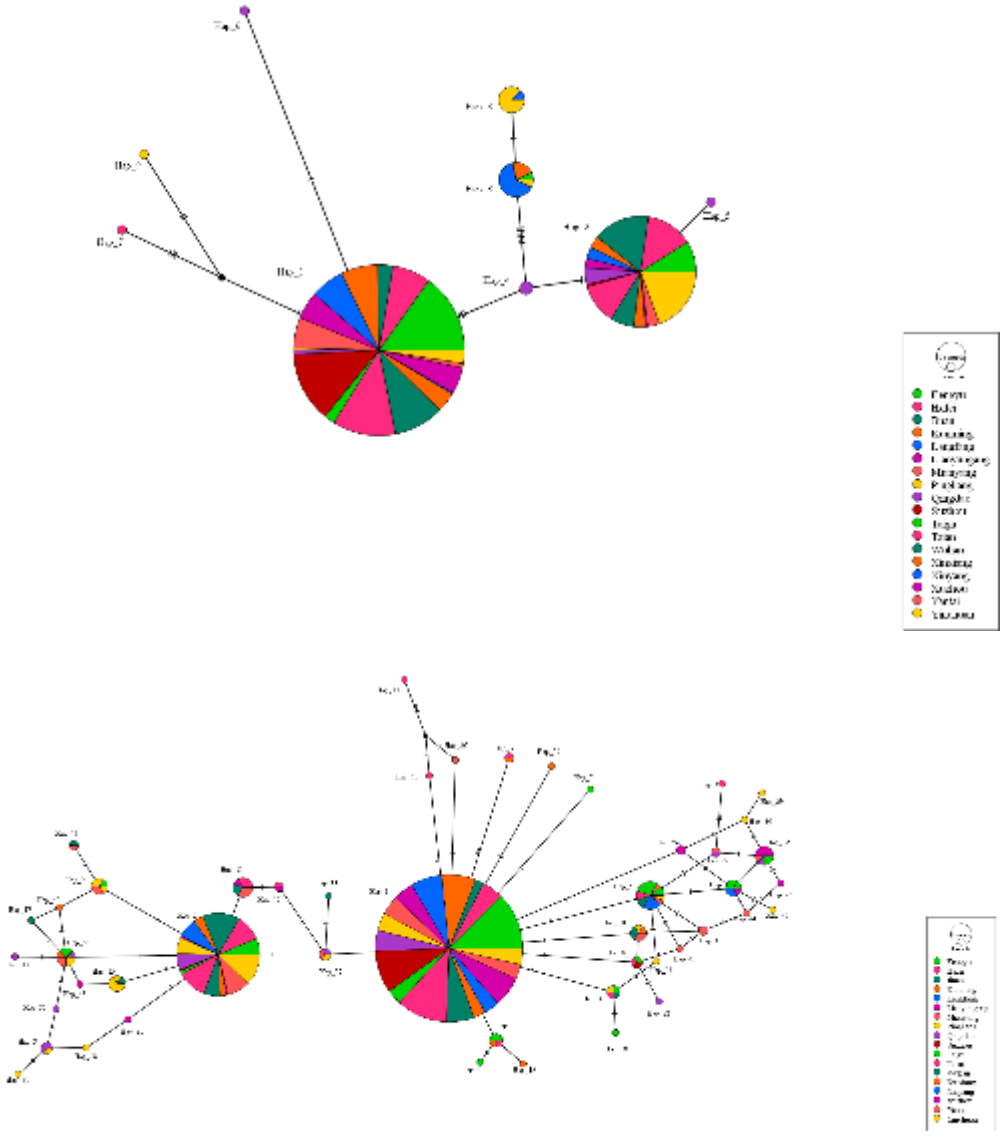
(A)



(B)



(C)



(D)

Figure 4-3. Haplotype network of *Sitobion miscanthi* from China based on *COI* (A), *EF-1α* (B), *gnd* (C), and *trpA* (D). Different colours represent different sampling locations.

The *EF-1α* network diagram (Figure 4-3B) showed that H3 included the greatest number of sequences and was an ancestral haplotype. H1 included the second largest number of sequences, and the sequences from Fengyu were mostly H1; thus, H1 was the dominant haplotype in Fengyu. In addition, Fengyu, Xuzhou, Suzhou, Taian, Yinchuan, Langfang and Pingliang all had unique haplotypes. The network map formed by the *gnd* gene haplotypes (Figure 4-3C) showed that H1 and H2 were located in the middle of the network map and formed the centres of two large star-shaped subnetworks. The remaining rare haplotypes were connected to H1 or H2 by mutations and were located on the edges of the network graph. The *trpA* network graph (Figure 4-3D) was also a large star-shaped network centred on haplotypes H1 and H2.

4.5.5. Historical population dynamics

When analyzing the historical dynamics of a population, neutrality testing and mismatch analysis are usually used. Neutrality test analysis uses parameter values to determine whether a population is a stable population or a recently formed population that has undergone expansion. Theoretically, in a stable population, the values of Fu's Fs and Tajima's D are close to 0. Significant negative value indicates that the population has undergone a sudden increase. Significant positive value indicates that the population may have differentiated, leading to the formation of a subpopulation, or that the population has undergone a bottleneck event. In mismatch analysis, when a population is a long-lived and stable population, the mismatch distribution map is usually multimodal; when a population is a recently formed or expanded population, the mismatch distribution map is usually unimodal.

When the historical dynamics of each population were analyzed separately, the Fu's Fs and Tajima's D values showed significant negative values, indicating that the populations had expanded. In the mismatch analysis, the mismatch distribution maps of *COI* and *gnd* showed double peaks, indicating a stable population.

4.6. Discussion

The process of species formation or biodiversity formation has always been an important subject in biological research. Speciation is a historical process that has occurred or is ongoing (Smith and Marcfoggin 1998), and the classification of species today is the result of evolution. However, observing ongoing speciation is difficult. Previous taxonomists have classified species by morphological markers (Gauthier *et al*. 2008), and doing so with large organisms is relatively feasible. However, for small creatures, insects and even microbes, this method does not work well. At present, biologists mainly use molecular genetic research methods to study such problems by examining molecular markers, such as mitochondrial sequences, nuclear genes, and microsatellites (Rémy *et al*. 1998; Nyakaana and Arctander 1999; Vignal *et al*. 2002). However, these molecular markers often evolve too slowly to clearly reveal the process of species differentiation. In the

past ten years, due to continuous research on endosymbiotic bacteria, the relationship between symbionts and aphids has attracted attention from many researchers. *Buchnera* belongs to the Enterobacteriaceae family of the Gammaproteo bacteria; it is the primary endosymbiotic bacterium of aphid insects and inhabits bacterial cells in the aphid body cavity (Nakabachi and Ishikawa 1999). Aphids and *Buchnera* have a strict symbiotic relationship, and these symbiotic bacteria provide a variety of essential amino acids for aphids (Wilkinson and Douglas 1996; Shigenobu *et al*. 2000; Birkle *et al*. 2002; Richards *et al*. 2010). Previous studies have found that the rate of synonymous mutations in the *Buchnera* genome is at least twice that in the mitochondrial genome (Liadouze *et al*. 1995; Douglas 1997; Sato and Ishikawa 1997; Douglas 1998; Bernays and Klein 2002). Given the history of the symbiotic relationship between aphids and *Buchnera*, it has been suggested that molecular markers in *Buchnera* could be used for genetic research in aphid populations. In this study, the genetic differentiation among aphid populations was analysed using the two endosymbiont *Buchnera* genes, *gnd* and *trpA*. Most populations' pairwise F_{ST} values based on *COI* and *gnd* were extremely differentiated. Only a few pairs had no genetic differentiation. The results provided strong support for the population diversity analysis based on mitochondrial and nuclear genes. Because of the higher number of polymorphic loci, the identification of symbiotic haplotypes was more refined, allowing a more accurate analysis of haplotypes among the populations. Comprehensive analysis of sequence polymorphism sites and genetic polymorphisms showed that genes from symbiotic bacteria could provide more genetic information for population differentiation studies than mitochondrial or nuclear genes alone.

China is a country with diverse geographical forms. The height of the terrain is distributed in three "steps" from west to east. Wheat is suitable for cultivation in temperate and subtropical climates, so wheat is mostly grown at two altitudes in eastern China (Xu *et al*. 2015). In terms of precipitation, wheat cultivation is performed in the monsoon zone in eastern China, where sufficient precipitation from the ocean can be obtained. Historically, wheat has been planted in China for more than 8,000 years. Since the Western Han Dynasty, wheat has been widely grown in the middle and lower reaches of the Yangtze River, the Huanghuaihai Basin, and Shandong, as these areas have sufficient water for wheat cultivation.

The life history of *S. miscanthi* shows that it can overwinter south of the 0 ℃ isotherm in January and survive in the north below 26 ℃ in July. The perennial habitat of *S. miscanthi* in China is also located in the Yunnan-Guizhou Plateau, the Sichuan Basin, the middle and lower reaches of the Yangtze River, the Huanghuaihai Basin, and the Jiaodong Peninsula. The study is based on the cluster analysis of the populations, which suggests that central China and the Jiaodong Peninsula are the main sources of *S. miscanthi* in China and that the Yunnan-Guizhou Plateau is a secondary source. The values of nucleotide diversity from Qingdao and Yantai were very high for the four genes. Wuhan and Xinxiang also had high nucleotide diversity value for the *COI* gene. Through cluster analysis of the haplotype sequences of *gnd*, we could see that Qingdao, Tai'an and Yinchuan

had unique haplotypes. The network diagram structure formed by the molecular markers of symbiotic bacteria was similar to the network diagram structure formed by mitochondria, and both were two main star-mounted structure network diagrams. This indicated that there may be two major population expansion events in the aphid population in China. Many small insects have also been found to be able to have a long-distance migration by air (Wei *et al*. 2015), and we could infer that these aphids appear first in the southwest and central regions and spread to the north with the help of the southeast and southwest monsoons in spring and summer. In autumn, they could spread to the south with the northeast and northwest monsoons.

However, the above inference is based on strong host specialization of *S. miscanthi* because it examines the genetic differentiation of 18 geographic populations. However, *S. miscanthi* has multiple hosts, and whether these populations are closely related to *S. miscanthi* populations on other hosts requires further study. In addition, the sample collection in this study was limited by time, some iconic populations (such as that in Tibet) were not collected, and there were certain limitations in estimating the aphid migration paths. We look forward to collecting and comparing additional populations of *S. miscanthi* from surrounding regions and conducting follow-up research.

4.7. Conclusions

Overall, we investigated the population genetic structure and demographic history of the *S. miscanthi* in China. This study revealed that majority of *S. miscanthi* populations had high genetic diversity. We infer that central China and the Jiaodong Peninsula are the main sources of *S. miscanthi* in China and that the Yunnan-Guizhou Plateau is a secondary source. *S. miscanthi* population control in the above areas should be strengthened to reduce insect reproduction at the sources and, thus, decrease aphid migration. Our research provides a successful example of a method for understanding the seasonal migration of insects. *S. miscanthi* populations in China expressed diverse reproduction patterns and significant genetic differentiation. The genetic differentiation in *S. miscanthi* populations is closely related to the geographic environment and climate; however, a geographic isolation was not observed.

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5

Chapter V: Contrasting population structure and demographic history of cereal aphids in different environmental and agricultural landscapes

From Morales-Hojas, R., **Sun, J**., Li, Q., Iraizoz, F.A., Tan, X., & Chen, J. 2020. Contrasting population structure and demographic history of cereal aphids in different environmental and agricultural landscapes. *Ecology and evolution*, 10, 9647-9662

5.1. Foreword

In the previous chapter, we speculated on the migration pathways of *S. miscanthi* in China. In this chapter, we studied the population genetic diversity and evolution of *S. miscanthi* and *S. avenae*, which are one of the most relevant grain aphids in Asia and Europe, respectively. We used genotyping by sequencing (GBS) to identify genome-wide single nucleotide polymorphisms (SNPs) to infer geographic structure and migration patterns. In this study, we show that the population structure of population is different from that described in previous studies, which suggests that their recent evolution may be a response to human-induced agricultural changes. This study shows that *S. avenae* in England is mainly parthenogenetic, and the population and spatial expansion of a single genetic cluster may correspond to the insecticide-resistant super clones identified in previous studies. On the contrary, *S. miscanthi* populations are mostly cyclical parthenogenesis in China. There is a sexual stage in autumn to produce overwintering eggs, and there are six genetic differentiation subgroups. The degree of genetic differentiation between geographical locations is high, which suggests that further taxonomical research is needed.

5.2. Abstract

Genetic diversity of populations has important ecological and evolutionary consequences, whose understanding is fundamental to improve the sustainability of agricultural production. Studies of how differences in agricultural management and environment influence the population structure of insect pests are central to predict outbreaks and optimize control programs. Here, we have studied the population genetic diversity and evolution of *Sitobion avenae* and *Sitobion miscanthi*, which are among the most relevant aphid pests of cereals across Europe and Asia, respectively. We have used genotyping by sequencing (GBS) to identify genome-wide single nucleotide polymorphisms (SNPs) to infer the geographic structure and migration patterns. In the present study, we show that the population structure in present-day populations is different from that described in previous studies, which suggest that they have evolved recently possibly as a response to human-induced changes in agriculture. This study shows that *S. avenae* in England is predominantly parthenogenetic and there has been a demographic and spatial expansion of a single genetic cluster, which could correspond with the insecticide resistance superclone identified in previous studies. Conversely, in China, *S. miscanthi* populations are mostly cyclical parthenogenetic, with one sexual stage in autumn to produce overwintering eggs, and there are six genetically differentiated subpopulations and high genetic differentiation between geographic locations, which suggests that further taxonomical research is needed. Unlike *S. avenae* in England, there is no evidence for insecticide resistance and there is no predominance of a single lineage in *S. miscanthi* in China.

5.3. Introduction

A major challenge in agricultural entomology is to develop efficient control strategies for pest organisms. For this, it is important to understand how environmental and anthropogenic factors influence the genetic structure and the evolutionary dynamics of insect populations (Pelissie, Crossley, Cohen, & Schoville, 2018). The level of genetic structure and diversity is the result of a combination of several factors which include selection, migration, and life history (i.e., reproduction mode) (Leffler et al., 2012), and studying their consequences on insect populations is of great interest to improve ecological agricultural practices (Pelissie et al., 2018). The use of pesticides remains a necessary way to control and manage pests in agriculture. However, their use imposes a strong selection pressure on pest populations and resistance to different types of insecticides has, therefore, evolved in many insects (Georghiou, 1972; Bass, Denholm, Williamson, & Nauen, 2015). Designing new strategies of pest control that rationalize the use of insecticides and reduce the likelihood of an evolution of resistance is key for the development of sustainable agriculture practices that reduce the environmental footprint (Wijnands, 1997), and understanding how pest populations respond to selective pressure and adapt to ecological changes is key to design rational strategies of management and control that are more targeted. In addition, a better understanding of the geographic connectivity between populations and the dispersal capacity of pests provides valuable information to control their abundance and distribution, while preventing also the spread of adaptive genetic variation, such as insecticide resistance, across their geographic range (Mazzi & Dorn, 2012). Therefore, it is essential that we incorporate the fundamental knowledge about population genetics into agricultural entomology.

Aphids comprise some of the most pernicious species of crop pests. In cereals, *Sitobion avenae* and *Sitobion miscanthi* are two of the most economically important species in Europe and Asia, respectively, and they are major vectors of the barley yellow dwarf virus (BYDV), which can severely reduce cereal yield (Vickerman & Wratten, 1979). Both *Sitobion* species are monoecious, nonhost-alternating, feeding only on Poaceae grasses and cereals (Blackman & Eastop, 2017). Like many aphids, *S. avenae* and *S. miscanthi* show different levels of variation in their life cycle, from individuals that are obligate cyclical parthenogenetic and have a generation that undergoes sexual reproduction in the primary host (holocycly), to clones that are obligate parthenogenetic and reproduce asexually all year round (anholocycly) (Dedryver, Le Gallic, Gauthier, & Simon, 1998). In addition, individuals can remain asexual in the cereal crops during winter as a response to environmental cues such as warmer temperatures and day length (Dedryver et al., 1998). As a result, a geographic cline in the reproductive type has been described in the *S. avenae* populations of UK and France, with increasing proportion of sexual reproduction toward the north of the countries (Simon et al., 1999; Llewellyn et al., 2003). In the case of *S. miscanthi*, variation in the life cycle has also been described. Populations from this species in Australia and New Zealand are anholocyclic, while they are holocyclic in

Taiwan (Sunnucks, England, Taylor, & Hales, 1996; Wilson, Sunnucks, & Hales, 1999). In China, *S. miscanthi* has been traditionally reported to be anholocyclic (Zhang, 1999; Guo, Shen, Li, & Gao, 2005). However, contrary to the observations in *S. avenae* and other species, a recent population genetics study has observed signatures of cyclical parthenogenesis in the southern populations of the country while obligate parthenogenetic reproduction would be dominant in the north (Wang, Hereward, & Zhang, 2016).

Resistance to pyrethroids was first detected in the UK populations of *S. avenae* in 2011. This was due to a knockdown resistance (*kdr*) mutation (L1014F) in the sodium channel gene (Foster et al., 2014). This knockdown appeared as a heterozygous mutation in one clone of *S. avenae*, known in the literature as clone SA3, and rapidly increased its abundance in the UK population from 2009 to 2014, although in variable proportions in different locations and years (Malloch, Williamson, Foster, & Fenton, 2014; Malloch, Foster, & Williamson, 2016; Dewar & Foster, 2017). The spread of the mutation in the UK was limited by the fact that the SA3 clone is anholocyclic, so pyrethroid resistance has not spread to other lineages through sexual recombination. In addition, the high connectivity of the UK populations revealed using four microsatellite loci (Llewellyn et al., 2003), probably facilitated the geographic spread of the resistant clone from its location of origin. Therefore, the continued use of pyrethroids combined with the long dispersal capacity of *S. avenae* has likely favored the spread of this clone across the UK. Nevertheless, the clonal diversity inferred using microsatellites remained high and similar to the diversity before the evolution of pyrethroid resistance, and other susceptible clones and phenotypes were still present in different proportions in British populations by 2015 (Llewellyn et al., 2003; Malloch et al., 2016). In the case of *S. miscanthi*, there is no available information in the literature regarding the evolution of insecticide resistance. However, understanding the dynamics and movement of the species can help manage and control the damage in cereal crops, and establish management programs to reduce the likelihood of insecticide resistance evolution. In China, previous studies have shown high levels of genetic diversity in *S. miscanthi* using a panel of five microsatellites (Guo et al., 2005; Wang et al., 2016), similar to those reported for *S. avenae* in the UK and France, and there is genetic differentiation between north and south of the country but low differentiation within each region, suggesting free gene flow within geographic regions (Guo et al., 2005; Wang et al., 2016).

In the present study, we analyze the population genetics and demographic history of *S. avenae* and *S. miscanthi* in England and China, respectively, using genotyping by sequencing (GBS) to identify potential weak differentiation. We discuss the results in view of the differences in life-history types and the evolution of insecticide resistance, which may be limited by the reproductive type. These genomics approaches have identified genetic variation at a national and regional scale for other aphids in regions where they disperse long distances (Morales-Hojas et al., 2019).

5.4. Material and methods

5.4.1. Samples

Individuals of *S. avenae* were collected using the network of 12.2 m high suction traps that is run by Rothamsted Insect Survey (RIS). The RIS suction traps are continuously collecting flying insects, and during the aphid season, aphid samples are identified daily to species level (Storkey et al., 2016; Morales-Hojas, 2017); of the identified aphids, 10 individuals of *S. avenae* collected during June–July 2018 with suction traps located in 12 sites across England (Starcross, Wye, Writtle, Broom's Barn, Kirton, Rothamsted, Silwood Park, Wellesbourne, Hereford, Preston, York, and Newcastle; see Table 1 and Figure 1) were used for this study. Individuals of *S. miscanthi* were collected in 10 sites (Kunming, Mianyang, Wuhan, Qingdao, Tai'an, Pingliang, Yinchuan, Langfang, Taigu, and Suzhou) across the cereal growing areas of China between February and June of 2017 (Table 5-1, Figure 5-1). The 10 individuals of *S. miscanthi* were collected from the same wheat field but from plants separated by 10 m to reduce the probability of sampling the same clone.

Table 5-1. Locations and number of samples (*N*) used in the present study

Country	Location	Geographic coordinates	*N*
UK	Broom's Barn (BB)	52.260681, 0.56843	10
UK	Hereford (H)	52.124201, −2.638156	10
UK	Kirton (K)	52.924454, −0.052153	10
UK	Newcastle (N)	55.213254, −1.685083	10
UK	Preston (P)	53.854383, −2.76699	10
UK	Rothamsted (RT)	51.806997, −0.360091	10
UK	Silwood Park (SP)	51.40941, −0.643357	10
UK	Starcross (SX)	50.629596, −3.45463	10
UK	Wellesbourne (We)	52.205975, −1.605017	10
UK	Writtle (Wr)	51.733599, 0.429233	10
UK	Wye (W)	51.185507, 0.944941	10

Country	Location	Geographic coordinates	*N*
UK	York (Y)	54.014616, −0.97320532	10
China	Kunming (KM)	24.8855, 102.8215	10
China	Mianyang (MY)	31.5347, 104.5676	10
China	Wuhan (WH)	30.5820, 114.0292	10
China	Qingdao (QD)	36.3074, 120.3963	10
China	Tai'an (TA)	36.1920, 117.1353	10
China	Pingliang (PL)	35.5426, 106.6748	10
China	Yinchuan (YC)	38.4731, 106.2428	10
China	Langfang (LF)	39.5031, 116.6857	10
China	Taigu (TG)	37.4212, 112.5513	10
China	Suzhou (SZ)	31.3023, 120.6313	10


Newcastle
Preston
York
Kirton
Hereford
Wellesbourne
Broom's Barn
Rothamsted
Writtle
Silwood Park
Wye
Starcross

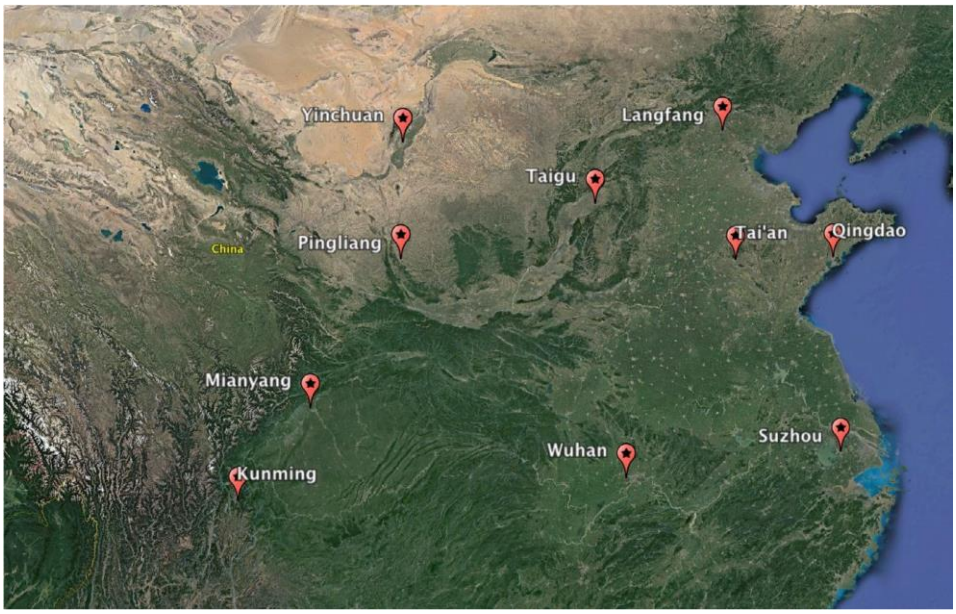


Figure 5-1. Maps showing the locations where samples of *Sitobion avenae* were collected in England (a) and where *Sitobion miscanthi* aphids were collected in China (b)

5.3.2. DNA extraction and SNP genotyping

DNA was extracted from samples using Qiagen's DNeasy Blood and Tissue kit following the manufacturer's protocol. Identification of SNP loci was done using GBS. Library preparation and sequencing of samples were outsourced commercially to Novogene LTD in the case of *S. avenae* and Allwegene Technology LTD *S. miscanthi*. Briefly, genomic DNA was digested with MseI in the case of *S. avenae* and with *ApeKI* in the case of *S. miscanthi*. The library preparation was performed following the standard Illumina pair-end (PE) protocol, and PE sequencing of 150 bp was performed on an Illumina HiSeq platform. Read quality was assessed with FastQC v0.67, and in the case of *S. miscanthi*, the first 10 bases were trimmed due to low quality using trimmomatic 0.36.1 (Bolger, Lohse, & Usadel, 2014). Reads were mapped to a draft of the *S. avenae* genome using BWA-MEM 0.7.16.0 letting BWA choosing the best algorithm to construct the index and the option of setting read group information Picard style. Duplicates were removed using MarkDuplicates v2.7.1.1, and indels were realigned with BamLeftAlign v1.0.2.29-1. Variant calling was carried out with FreeBayes v1.0.2.29-3 (Garrison & Marth, 2012) with a simple diploid calling of variants (standard filters of minimum mapping quality of 30, minimum base quality of 30, default minimum supporting allele qsum, and genotype variant threshold) and minimum coverage of 2. The resulting SNPs from FreeBayes were annotated using snpEff v4.0. These tools were run using Galaxy v17.05 (Afgan et al., 2016)

and parameters provided refer to the Galaxy options. SNPs called with FreeBayes were filtered using VCFtools v0.1.14 (Danecek et al., 2011) before the markers were used in subsequent analyses. Different filtering schemes were tested in each species to obtain a dataset that maximized the quality of the SNPs and genotypes while minimizing the missing data at marker and individual levels, as recommended by O'Leary, Puritz, Willis, Hollenbeck, and Portnoy (2018). Given that GBS was performed separately for the two species, the best filtering schemes were different (vcftools parameters *S. miscanthi*: max-missing 0.75, minDP 3, mac 3, minQ 30, remove-indels, thin 2000, max-missing 0.9, thin 5,000; S. avenae: max-missing 0.5, mac 3, minQ 30, minDP 3, max-missing 0.5, exclude individuals with 50% missing data, max-missing 0.75, remove-indels, thin 2000).

5.3.3. Analysis of population structure

The population structure of both species was investigated using the Bayesian genetic clustering algorithm implemented in Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). We used the admixture model with correlated frequencies, and to detect any potential subtle genetic structure, we ran Structure with the sampling locations set as priors (locprior = 1); this model has the power to detect a weak structure signal and does not bias the results toward detecting genetic structure when there is none. Analyses for the two species were performed separately and with different parameters as the datasets were obtained following different protocols (different sequencing companies) and differed in number of individuals, markers, and quality. In the case of *S. miscanthi*, a first run of Bayesian clustering analyses of the population structure was carried out with five independent simulations with 100,000 burn-in and 100,000 mcmc chains for each of *K* 1–10. An additional run of five independent simulations with 100,000 burn-in and 500,000 mcmc chains was carried out for *K* 5–10 to confirm the results of the first run and ensure convergence in the mcmc step. In the analyses of *S. avenae* from the UK, Structure was run with 5 replicates of 500,000 burn-in and 1,000,000 mcmc chains for *K* ranging from 1 to 12. Summary statistics (alpha and likelihood parameters) convergence was inspected visually to confirm that the burn-in and run lengths were adequate. We ran the Structure simulations using a multicore computer with the R package ParallelStructure (Besnier & Glover, 2013) in the CIPRES science gateway server (Miller, Pfeiffer, & Schwartz, 2010). The number of K groups that best fitted the dataset was estimated using the method of Evanno, Regnaut, and Goudet (2005) using Structure Harvester Web v0.6.94 (Earl & Vonholdt, 2012). Cluster assignment probabilities were estimated using the program Clumpp (Jakobsson & Rosenberg, 2007) as implemented in the webserver CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015). To validate the identified number of clusters identified by Structure, the nonmodel-based method of discriminant analysis of principal components (DAPC) was performed as implemented in the R package adegenet 2.1.2 (Jombart, 2008; Jombart, Devillard, & Balloux, 2010; Jombart & Ahmed, 2011). The number of genetic clusters was identified using the Bayesian information criterion (BIC) with the “find.clusters” function in adegenet 2.1.2 with manual and automatic

selection (choose.n.clust = FALSE) with the good fit criterion to choose *K*.

The genetic diversity measures of the populations were estimated using Arlequin 3.5.2.2 (Excoffier, Laval, & Schneider, 2005); Fis was also estimated and tested for using the exact probability test in the R version of Genepop v. 4.7.5 (Rousset, 2008). Genetic variation among populations was investigated using an analysis of the molecular variance (AMOVA) with 10,000 permutations using Arlequin 3.5.2.2. We used hierarchical AMOVA to test the population structures resulting from the Structure runs. Population pairwise divergence was investigated using F_{ST}, and the significance was evaluated with 10,000 permutations in Arlequin. We ran Mantel tests as performed in Arlequin to evaluate the correlation between the genetic distances (F_{ST}) and the geographic distance between sampling locations. The geographic distances (in Km) were estimated using Google maps (Tables 5 and 10). The demographic history was also explored using Arlequin. For this, we first estimated the gametic phase from the multilocus diploid data using the ELB algorithm with the default parameter values in Arlequin 3.5.2.2 (Excoffier, Laval, & Balding, 2003). Population expansion or bottleneck was inferred using Fu's FS (Fu, 1997), and mismatch analyses were run with 1,000 bootstrap replicates to estimate Harpending's raggedness index and the sum of squared deviation (SSD) given the population expansion and spatial expansion models.

Phylogenetic trees were constructed using maximum likelihood (ML) with RAxML 8.2.12 (Stamatakis, 2014) run in the server CIPRES (Miller et al., 2010). The data matrix used was the phased haplotypes. RAxML was run with 1,000 bootstrap inferences with subsequent ML search using the gtrgamma model. The Lewis correction for ascertainment bias was implemented as it is the appropriate model for binary datasets that include only variable sites (as it is the case of SNPs) (Lewis, 2001; Leache, Banbury, Felsenstein, de Oca, & Stamatakis, 2015). Phylogenetic trees have been visualized and edited in FigTree v1.4.2.

5.4. Results

5.4.1 Genetic diversity and population structure of Sitobion miscanthi in China

A total of 14,520 SNPs with less than 20% of missing data per individual (0.4%-18%, 5% average) and 10% per locus (0%-9%, 2% average) were obtained for the 100 individuals from the 10 Chinese populations. Of these loci, approximately 4% deviated from the Hardy–Weinberg equilibrium (HWE) after Bonferroni correction. The levels of gene diversity (H_e) observed across all populations are lower than in previous studies of *S. miscanthi* in China, but similar to that of the UK population of *S. avenae* and other cereal aphids like *Rhopalosiphum padi* (Morales-Hojas et al., 2019) (Table 5-2). Overall, the Chinese population is not in HWE, and the inbreeding coefficient is positive (Table 5-2). The same positive, significant F_{IS} is observed when we group the populations into North China (Qingdao, Tai'an, Langfang, Taigu, Pingliang, and Yinchuan) and South China

(Shuzou, Wuhan, Mianyang, and Kunming) following the Qinglin–Huaihe line (QHL; the traditional identified geographic north–south divide of China) (Table 5-2). Furthermore, several populations in China (Qingdao, Taigu, Yinchuan, and Wuhan) are also not in HWE, with significant, positive F_{IS} indices. Significant, positive estimates of F_{IS} are generally the result of inbreeding in the population or the effect of population subdivision, the Wahlund effect.

Table 5-2. Mean genetic diversity indices estimated for each *Sitobion miscanthi* population, populations north and south of the QHL, and each of the identified genetic clusters (GC)

	H_e	H_o	F_{IS}	p(random F_{IS} ≥ observed F_{IS})
Overall	0.28933	0.16720	0.3871	.0000
North	0.33122	0.19451	0.37625	.0000
Qingdao	0.3796	0.30129	0.16357	.0117
Tai'an	0.46466	0.44985	−0.01467	.5147
Langfang	0.42943	0.40584	0.00621	.4766
Taigu	0.2948	0.22396	0.21015	.0407
Pingliang	0.41717	0.34735	0.08827	.1097
Yinchuan	0.30452	0.22792	0.24868	.0344
South	0.30482	0.19169	0.33871	.0000
Shuzou	0.36834	0.313	0.10903	.0689
Wuhan	0.3134	0.19447	0.36651	.0004
Mianyang	0.46364	0.4364	−0.00632	.4827
Kunming	0.35638	0.33113	0.05435	.2473
GC1	0.46035	0.49644	−0.10550	.8083
GC2	0.40616	0.35361	0.07863	.0447
GC3	0.45541	0.42329	−0.01105	.5731
GC4	0.41227	0.37039	0.05263	.1626

GC5	0.36980	0.38917	−0.08655	.8816
GC6	0.42755	0.43997	−0.05740	.5807

Note: H_o and H_e are observed and expected (gene diversity) heterozygosity, respectively; F_{IS}-inbreeding coefficient.

A first run of Bayesian clustering analyses of the population structure was carried out with five independent simulations with 100,000 burn-in and 100,000 mcmc chains for each of *K* 1–10. Analyses of the results following the Evanno method (Evanno et al., 2005) indicated that the most likely number of clusters was $K = 6$. An additional run of five independent simulations with 100,000 burn-in and 500,000 mcmc chains carried out for *K* 5-10 confirmed that the most likely number of *K* was 6 (Table 5-3, Figure 5-2). The structure plot shows that most sampled locations are not homogeneous, comprising individuals that are assigned to different genetic clusters (Figure 5-2). The genetic cluster 1 (GC1) comprises only individuals from Kunming KM1, KM2, KM3, KM4, KM5, KM6, KM7, KM9, and KM10; GC2 includes one individual from Kunming (KM8), all individuals from Langfang and Mianyang, two individuals from Pingliang (PL5 and PL10), one individual from Qingdao and Suzhou (QD6 and SZ7), and two from Wuhan (WH9 and WH10); GC3 comprises only individuals from Wuhan (WH1, WH2, WH3, WH4, WH7, and WH8); GC4 includes nine samples from Taigu (TG1, TG3, TG4, TG5, TG6, TG7, TG8, TG9, and TG10) and eight from Yinchuan (YC1, YC2, YC3, YC4, YC5, YC6, YC7, and YC9); GC5 is comprised by nine individuals from Qingdao (QD1, QD2, QD3, QD4, QD5, QD7, QD8, QD9, and QD10), all samples from Tai'an, one from Suzhou (SZ1), and two from Wuhan (WH5 and WH6); and GC6 includes eight samples from Pingliang (PL1, PL2, PL3, PL4, PL6, PL7, PL8, and PL9), eight from Suzhou (SZ2, SZ3, SZ4, SZ5, SZ6, SZ8, SZ9, and SZ10), one from Taigu (TG2), and two from Yinchuan (YC8 and YC10). These results suggest that the Wahlund effect, the mixture of genetically different populations, is the most likely reason for the significant F_{IS} in Qingdao, Taigu, Yinchuan, and Wuhan. This is further supported by the fact that F_{IS} is nonsignificant when the individuals are grouped according to the genetic cluster to which they were assigned by the Bayesian clustering analyses with Structure, and thus, genetic clusters are in HWE except GC2 ($F_{IS} = 0.07863$, $p = .0447$) that includes 27 individuals from seven different locations (Table 5-2).

Table 5-3. Table of results from Structure for the Chinese populations (a) five independent simulations for *K* 1-10, 100,000 burn-in and 100,000 mcmc chains; (b) five independent simulations for *K* 5-10, 100,000 burn-in and 500,000 mcmc chains

K	Reps	Mean Ln*P*(*K*)	Stdev Ln*P*(*K*)	Ln′(*K*)	|Ln″(*K*)|	Delta *K*
(a)						
1	5	−1,237,847.48	14.9028	NA	NA	NA
2	5	−1,068,686.16	8,702.18	169,161.32	75,998.68	8.733291
3	5	−975,523.52	35,417.94	93,162.64	25,385.46	0.716740
4	5	−856,975.42	29,834.73	118,548.10	133,411.76	4.471693
5	5	−871,839.08	51,758.53	−14,863.66	35,760.94	0.690919
6	5	−850,941.80	35,376.44	20,897.28	68,821,957.12	1,945.417782
7	5	−69,652,001.64	140,217,321.35	−68,801,059.84	137,586,196.24	0.981235
8	5	−866,865.24	73,746.76	68,785,136.40	76,730,695.74	1,040.461790
9	5	−8,812,424.58	17,564,554.27	−7,945,559.34	15,848,078.50	0.902276
10	5	−909,905.42	61,354.10	7,902,519.16	NA	NA
(b)						
5	5	−962,395	53,822.58	NA	NA	NA
6	5	−949,885	94,126.26	12,510.06	49,025,850	520.852
7	5	−5E + 07	49,016,699	−4.9E + 07	55,801,634	1.138421
8	5	−4.3E + 07	59,145,788	6,788,294	28,389,784	0.479997
9	5	−6.5E + 07	41,036,977	−2.2E + 07	59,738,205	1.455717
10	5	−2.7E + 07	23,812,471	38,136,715	NA	NA

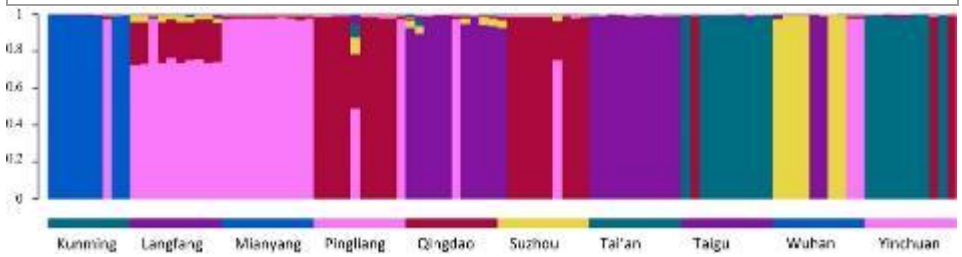


Figure 5-2. Structure analysis based on 14,520 SNPs across 10 Chinese populations, with $K = 6$. Each bar represents one individual and the colors of the bars the posterior

probability that each belongs to one of the six genetic clusters. GC1—blue; GC2—magenta; GC3—yellow; GC4—green; GC5—purple; GC6—red

Analysis of the molecular variance (AMOVA) indicated that the overall F_{ST} of *S. miscanthi* in China was 0.3254 (p = 0); thus, 32.54% of the total genetic variation was explained by differences between the populations. When the individuals were grouped according to their assigned genetic cluster, 40.68% of the genetic variation (F_{CT} = 0.4069, p = 0) was explained by differences among the groups (Table 4). Finally, we tested the QHL north–south subdivision of the *S. miscanthi* population previously suggested in the literature with an AMOVA. Results did not support the QHL divide hypothesis with only a nonsignificant 2.22% of the genetic variation being explained by such geographic division (F_{CT} = 0.0222, p = .2569) (Table 5-4).

Table 5-4. AMOVA of the SNP dataset from *Sitobion miscanthi*

	Source of variation	Sum of squares	Variance components	% variation	Fixation indices
A	Among groups	146,960.625	840.81437	40.68	F_{CT} = 0.4068 (p = 0)
	Among populations within groups	23,445.277	83.52677	4.04	F_{SC} = 0.0681 (p = 0)
	Among individuals within populations	90,706.443	−22.79868	−1.10	F_{IS} = −0.0199 (p = .6556)
	Within individuals	116,543.000	1,165.43000	56.38	F_{IT} = 0.4362 (p = 0)
B	Among groups	17,982.662	43.99279	2.22	F_{CT} = 0.0222 (p = .2569)
	Among populations within groups	110,074.833	614.04835	31.01	F_{SC} = 0.3172 (p = 0)
	Among individuals within populations	133,054.850	156.47861	7.90	F_{IS} = −0.1184 (p = 0)
	Within individuals	116,543.000	1,165.43000	58.86	F_{IT} = 0.4114 (p = 0)

Note: Analyses were performed to test the following hierarchical substructure: (A) individuals grouped according to the genetic cluster assignment from Structure; (B) populations grouped according to their north–south location with respect to the Qinling–Huaihe line divide.

Pairwise F_{ST} tests showed that the genetic differentiation between the different populations is high and significant in most cases (Table 5-5). The only exceptions are between the populations of Suzhou and Pingliang, and Yinchuan and Taigu, which showed no genetic differentiation. Qingdao and Tai'an, which are separated by 300 km, showed a low but significant F_{ST}, and Langfang, Pingliang, Mianyang, and Suzhou showed an intermediate and significant F_{ST}, despite some of these locations being more than 1,000 km apart. The genetic differentiation between the genetic clusters is higher and significant in all pairwise comparisons (Table 5-6).

Table 5-5. Genetic differentiation between populations estimated with pairwise F_{ST} (below the diagonal) with significant values (p value < .001 after the exact test estimated with 10,100 permutations) in italics; geographic distances between samples in kilometers above the diagonal

	South populations				North populations					
	Kunming	**Mianyang**	**Wuhan**	**Suzhou**	**Yinchuan**	**Pingliang**	**Taigu**	**Tai'an**	**Langfang**	**Qingdao**
Kunming		750	1,287	1,872	1,546	1,287	1,668	1,875	2,120	2,095
Mianyang	0.38556		925	1,507	792	534	980	1,276	1,430	1,540
Wuhan	0.35288	0.25497		598	1,156	940	785	710	1,015	827
Suzhou	0.35113	0.13993	0.20726		1537	1,394	1,010	666	980	530
Yinchuan	0.47602	0.36352	0.48536	0.31532		348	564	990	934	1,279
Pingliang	0.36378	0.12852	0.2295	−0.00006	0.3171		550	947	980	1,242
Taigu	0.52138	0.43957	0.5479	0.39557	−0.01011	0.40377		426	450	704
Tai'an	0.40604	0.37101	0.23802	0.29632	0.46615	0.33196	0.52301		390	300
Langfang	0.37918	0.13704	0.26178	0.11658	0.34895	0.12602	0.42251	0.35654		508
Qingdao	0.34611	0.27895	0.17379	0.2091	0.40618	0.24716	0.4634	0.05756	0.2752	

Table 5-6. Genetic differentiation (pairwise F_{ST}) between the six genetic clusters (GC) identified with Structure

	GC 1	**GC 2**	**GC 3**	**GC 4**	**GC 5**	**GC 6**
Genetic cluster 1	0.00000					
Genetic cluster 2	0.42822	0.00000				
Genetic cluster 3	0.46296	0.13319	0.00000			
Genetic cluster 4	0.43198	0.30765	0.32792	0.00000		
Genetic cluster 5	0.65910	0.45260	0.51381	0.54811	0.00000	
Genetic cluster 6	0.55366	0.40099	0.44521	0.38811	0.79219	0.00000

Note： All pairwise comparisons were significant (p = 0) as estimated with 10,100 permutations. GC 1 includes only individuals (n = 9) from Kunming; GC 2 comprises individuals from Kunming (1), Langfang (10), Mianyang (10), Pingliang (2), Qingdao (1), Suzhou (1), and Wuhan (2); GC 3

consists of individuals from Wuhan (6); GC 4 includes individuals collected in Taigu (9) and Yinchuan (8); GC 5 is formed by individuals from Qingdao (9), Suzhou (1), Tai'an (10), and Wuhan (2); and GC 6 includes individuals from Pingliang (8), Suzhou (8), Taigu (1), and Yinchuan (2).

The Mantel test was nonsignificant when no genetic structure within *S. miscanthi* is considered (slope 0.000044, $R = 0.1514$, $p = .205$) rejecting the isolation by distance (IBD) hypothesis. However, as the Bayesian clustering analyses showed, there are 6 likely genetic clusters and this subdivision of the population can bias the test. To control for this, we performed Mantel tests for each of the identified clusters 2, 5, and 6 separately (these clusters included individuals from more than one location) but results were still nonsignificant (GC 2: slope −0.000022, R = −0.1496, p = .719; GC5: slope −0.000016, R = −0.0387, p = .538; GC6: slope 0.000036, R = 0.7177, p = .118). Nevertheless, the low number of individuals for some of the locations within each cluster limits the statistical power of the tests.

Phylogenetic analysis of *S. miscanthi* SNP dataset resulted in a phylogeny that reflected the inferred population structure (Figure 5-3). The evolutionary tree shows six main clades with high bootstrap support corresponding to the identified genetic clusters. The relationships between the clades are also well supported, and they provide further information regarding their evolutionary relationship. Thus, the Kunming population (Genetic Cluster 1) is the sister clade to a supergroup comprising the other clades. Haplotypes from Tai'an and Qingdao (GC5), which are geographically closer to each other than to other locations, cluster together with 4 haplotypes from two individuals collected in Wuhan and two haplotypes from Suzhou; the sister group to this clade comprises only samples from Wuhan (GC3), despite being geographically distant. The sister clade to this clade comprising these two clusters includes the genetic clusters 2, 4, and 6; within this clade, GC6 comprising individuals from Yinchuan, Pingliang, Suzhou, and Taigu is the sister group to GC4 and GC2, which include individuals from most geographic locations. Overall, there is no geographic signature observed in the phylogenetic tree in accordance with the population structure analyses, except for a clustering of the majority of the individuals from Kunming in a separate clade.

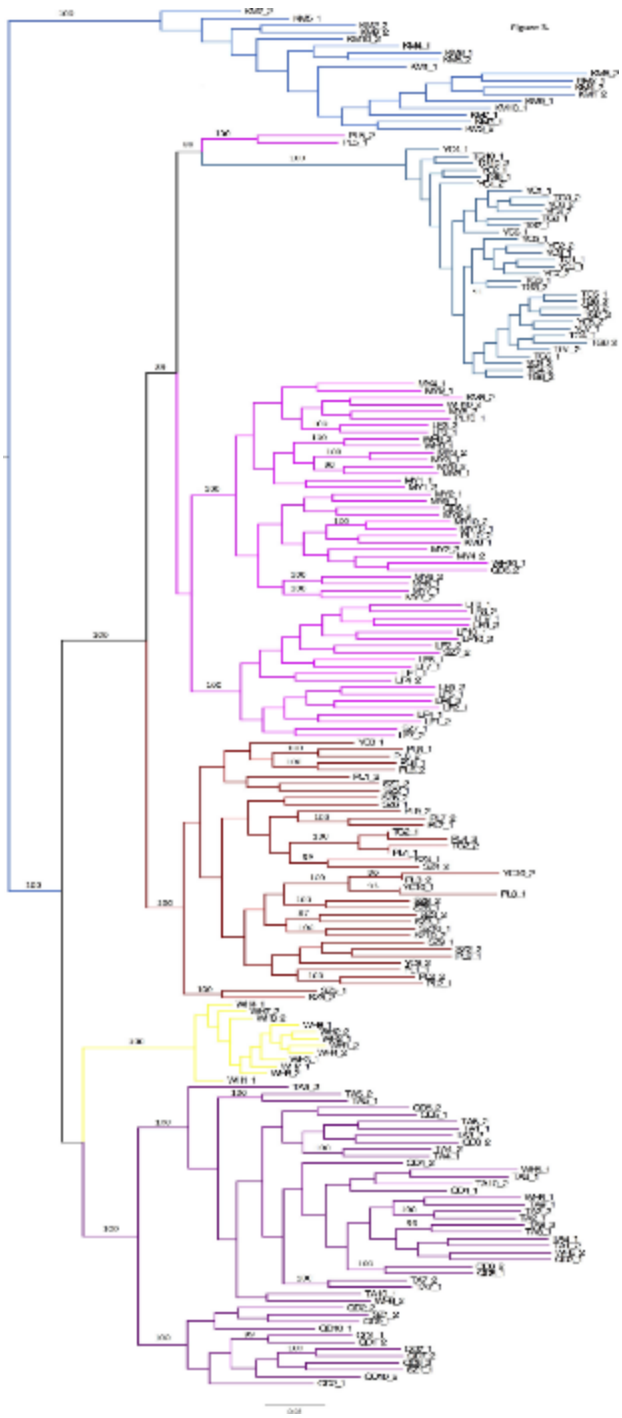


Figure 5-3. Midpoint rooted phylogenetic tree estimated with RAxML for the *Sitobion miscanthi* phased haplotypes from China using a dataset of 14,520 SNPs. The six genetic clusters are highlighted with different colors, corresponding to the colors in the bar plot. Labels on branches are bootstrap values >90%. GC1—blue; GC2—magenta; GC3—yellow; GC4—green; GC5—purple; GC6—red

5.4.2. Genetic diversity and population structure of Sitobion avenae in the UK

A total of 846 SNPs with less than 25% of missing data per locus (0%-24%, 13% mean) and <50% per individual (2%-48%, 14% mean) were identified in 98 individuals from 12 sampling locations across England. Approximately 19% of these loci are deviated from the HWE after Bonferroni correction. The gene diversity, estimated as the He, is high across all populations (Table 7), although it is lower than the He observed in a previous analysis of the UK populations using four microsatellites (Llewellyn et al., 2003), although comparison of the results obtained with genome-wide markers obtained in the present analysis and that of microsatellites has to be done with care. The H_o was higher in all populations compared to the H_e, resulting in high negative F_{IS} estimates which indicate that the *S. avenae* in England is not in HWE (Table 5-7). This contrasts with the situation of the UK population 15–20 years ago where most markers in the analyzed populations were in HWE (Llewellyn et al., 2003). Although the negative F_{IS} values were not significant according to the permutation test performed by Arlequin 3.5, it should be noted that this test evaluates the probability of obtaining random values that are higher than the observed ones rather than the probability of random values being more negative. Therefore, it is most probable that the *p* values reported are not correct for these values. When F_{IS} were estimated using Genepop, all were also negative and most were significant using the exact test. The negative F_{IS} is the result of an excess of heterozygotes, and it is considered a signature of clonal reproduction.

Table 5-7. Mean genetic diversity indices estimated for each *Sitobion avenae* population in England and overall

	N	***He***	***Ho***	***F*IS (Arlequin)**	***F*IS (Genepop)**
Overall	196	0.28433	0.41243	−0.6928	
Broom's Barn	20	0.37289	0.54525	−0.6631	−0.5101**
Hereford	20	0.37214	0.53194	−0.8069	−0.4745**
Kirton	18	0.38078	0.54714	−0.7750	−0.4908*
Newcastle	20	0.38088	0.56614	−0.7302	−0.5401**
Preston	12	0.40722	0.57489	−0.7154	−0.4879
Rothamsted	16	0.37388	0.51535	−0.6035	−0.4271
Silwood Park	14	0.39285	0.54213	−0.7222	−0.4382

Starcross	2	–	–	–	–
Wye	18	0.36667	0.51283	−0.7137	−0.4460
Wellesbourne	20	0.35591	0.50472	−0.6003	−0.4594**
Writtle	18	0.36290	0.51392	−0.6405	−0.4660**
York	18	0.37310	0.53559	−0.7419	−0.4913**

Note: *N* is the number of gene copies (2 × number of individuals); *H*o and *H*e are observed and expected (gene diversity) heterozygosity, respectively; F_{IS}—inbreeding coefficient. Starcross values are not included as it was represented by one single individual.

Bayesian clustering analysis was run with 5 replicates of 500,000 burn-in and 1,000,000 mcmc chains to ensure convergence. Results were analyzed following the Evanno method (Evanno et al., 2005), which suggested that the most likely number of genetic clusters is $K = 2$ (Table 5-8). However, this method does not estimate the deltaK for $K = 1$ and the mean LnP(K) is maximized for $K = 1$ suggesting that the most likely number of clusters is 1 (Table 5-8). In addition, the standard deviation increases from $K = 2$, which usually happens after reaching the best K. Finally, there is no clear distribution of individuals into 2 differentiated clusters in the bar plot resulting from the analyses with $K = 2$, with all of them having some probability of belonging to each cluster (Figure 5-4). An additional nonmodel-based analysis to verify the number of clusters identified by Structure in the data was performed with an unsupervised clustering approach (DAPC) (Jombart et al., 2010). For this, the "find.clusters" function of the R package adegenet 2.1.2 was used (Jombart, 2008; Jombart & Ahmed, 2011). This function applies a DAPC method to identify the optimal number of clusters using a model selection criterion, in this case the Bayesian information criterion (BIC). The BIC is minimized when $K = 3$ (BIC = 384.6787) but the difference with $K = 1$ (BIC = 386.0667) is of 1.388 and differences smaller than 2 between two models are considered to be weak evidence (Raftery, 1995). When automatic selection was applied using the good fit criterion, the number of optimal clusters retained was $K = 1$. Taking all the results in consideration, they suggest that there is no population structure but just one genetic cluster.

Table 5-8. Table of results from structure for the aphid English populations (five independent simulations for K 1–12, 500,000 burn-in and 1,000,000 mcmc chains)

K	**Reps**	**Mean Ln*P*(*K*)**	**Stdev Ln*P*(*K*)**	**Ln′(*K*)**	**|Ln″(*K*)|**	**Delta *K***
1	5	−55,651.5	0.8927	NA	NA	NA
2	5	−59,437.7	795.9415	−3,786.2	4,110.34	5.164123
3	5	−67,334.3	1,512.473	−7,896.54	4,447.6	2.940614
4	5	−70,783.2	4,688.426	−3,448.94	3,568.64	0.761159
5	5	−70,663.5	3,032.39	119.7	6,005.16	1.980339

6	5	−76,549	5,716.411	−5,885.46	12,612.16	2.206308
7	5	−69,822.3	2,700.041	6,726.7	9,361.38	3.467125
8	5	−72,456.9	7,835.535	−2,634.68	7,552.04	0.963819
9	5	−67,539.6	3,157.942	4,917.36	4,039.52	1.279162
10	5	−6,6661.7	4,041.049	877.84	2,479.22	0.613509
11	5	−63,304.7	2,208.609	3,357.06	5,218.48	2.362791
12	5	−65,166.1	3,124.338	−1,861.42	NA	NA

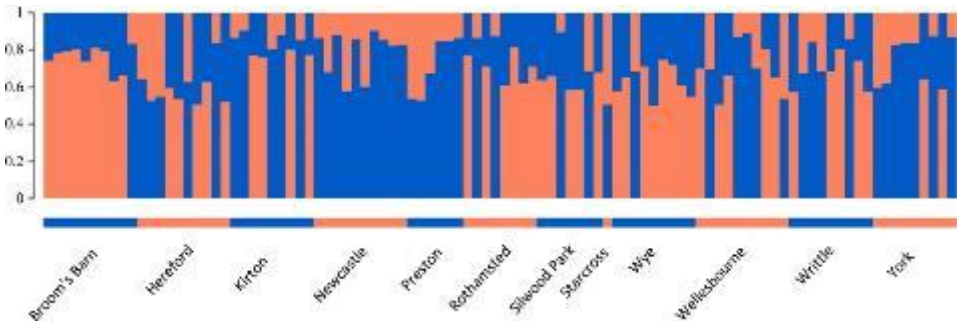


Figure 5-4. Structure analysis based on 846 SNPs across 12 English populations of *Sitobion avenae* for $K = 2$. Each bar represents one individual and the colors of the bars the posterior probability that each belongs to each of the genetic clusters

Analyses of the molecular variance (AMOVA) also supported the existence of one single gene cluster. The overall diversity of *S. avenae* in England was low $F_{ST} = 0.001$ and nonsignificant among populations, indicating a low differentiation level. When the individuals were clustered according to the Structure results for $K = 2$, assigning individuals to each genetic cluster according to the membership coefficient estimated with clump, the amount of genetic variation that was explained between groups was 2.33% ($p = 0$) (Table 5-9). These results support those of Structure with one single genetic cluster and no population structure. In addition, the genetic differentiation estimates (F_{ST}) were all negative (Table 5-10), which indicates that there is no divergence between the different locations. Similarly, when the two possible genetic clusters identified with Structure are compared, the F_{ST} is low (0.0158; $p = 0$), further supporting the lack of population structure in *S. avenae* from England. Mantel test rejected the IBD hypothesis (slope 0, R = n.a., $p = 1$) as expected given the lack of genetic differentiation between locations (F_{ST}).

Table 5-9. AMOVA of the SNP dataset from *Sitobion avenae*

	Source of variations	Sum of squares	Variance components	% variation	Fixation indices
A	Among groups	203.571	1.87014	2.33	F_{CT} = 0.0233 (p = 0)
	Among populations within groups	397.908	−0.21891	−0.27	F_{SC} = −0.0028(p = .8146)
	Among individuals within populations	1,765.164	−55.77004	−69.41	F_{IS} = −0.7087 (p = 1)
	Within individuals	13,177.500	134.46429	167.36	F_{IT} = −0.6736 (p = 1)

Note： Analyses were performed to test the genetic cluster assignment from Structure, K = 2.

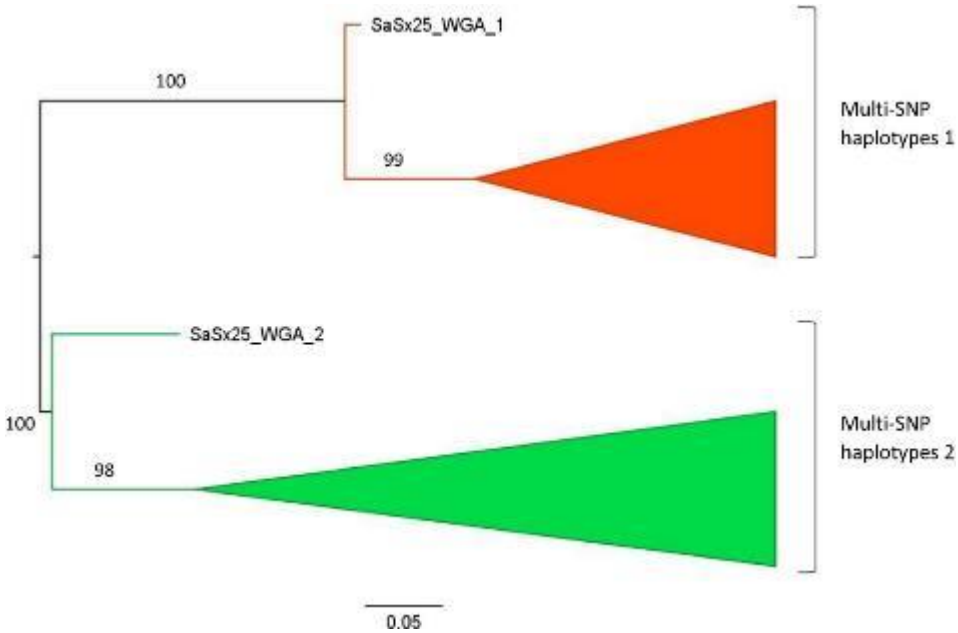
Table 5-10. Genetic differentiation between populations estimated with pairwise F_{ST} with significant values (p value < .001 after the exact test estimated with 10,100 permutations) in italics; geographic distances between samples in kilometers above the diagonal

	BB	H	K	*N*	P	RT	SP	SX	W	We	Wr	Y
BB	–	217	79	358	286	80	124	335	124	148	59	222
H	−0.0195	–	197	350	195	160	155	176	268	71	215	240
K	−0.0311	−0.0297	–	275	206	121	170	347	205	130	136	138
N	−0.0051	−0.0244	−0.0438	–	161	384	425	524	480	331	410	137
P	−0.0206	−0.0454	−0.0590	−0.0499	–	277	304	363	387	195	319	119
RT	−0.0321	−0.0410	−0.0466	−0.0281	−0.0433	–	50	253	111	97	54	250
SP	−0.0292	−0.0471	−0.0419	−0.0374	−0.0528	−0.0549	–	213	112	113	83	294
SX	−0.4919	−0.4619	−0.4359	−0.5208	−0.5768	−0.5711	−0.4966	–	314	220	298	415
W	−0.0329	−0.0328	−0.0420	−0.0269	−0.0430	−0.0532	−0.0470	−0.4958	–	208	71	340
We	−0.0162	−0.0460	−0.0461	−0.0301	−0.0447	−0.0408	−0.0515	−0.5804	−0.0427	–	150	205
Wr	−0.0243	−0.0420	−0.0501	−0.0362	−0.0483	−0.0454	−0.0507	−0.5559	−0.0451	−0.0395	–	272
Y	−0.0139	−0.0273	−0.0462	−0.0449	−0.0515	−0.0405	−0.0444	−0.5112	−0.0254	−0.0406	−0.0406	–

Abbreviations: BB, Broon's Barn; H, Hereford; K, Kirton; N, Newcastle; P, Preston; RT, Rothamsted; SP, Silwood Park; SX, Starcross; W, Wye; We, Wellesbourne; Wr, Writtle; Y, York.

Phylogenetic analyses were run using the two multi-SNP haplotypes of each individual. The inferred ML phylogeny comprised two major clades highly supported by bootstrap (Figure 5-5). Each of these two clades included one of the multi-SNP haplotypes from each individual, so that each multi-SNP haplotype is more closely related to a haplotype from another individual than to the second haplotype from the same individual. This type of phylogenetic topology can be the result of asexual reproduction from a single individual, in which all copies from each of the extant haplotypes derive from one common ancestral haplotype with no recombination. The basal node would represent the common ancestral asexual individual. Thus, the phylogeny supports the lack of population structure and suggests that there is one single clone or genetic cluster dominating the English population of *S. avenae*, and the asexual reproduction of this lineage.

Figure 5-5. Midpoint rooted phylogenetic tree estimated with RAxML for *Sitobion avenae* phased haplotypes from England using a dataset of 846 SNPs. The two-phased multimarker haplotypes from every individual are colored in red and green, and the clades have been collapsed except for the earliest branching haplotype of each clade. Labels on branches are bootstrap values >90%.



Analyses of the historical demography of this species in England showed a population and spatial expansion of the genetic cluster identified in the population genetic structure analysis, which would be consistent with the increase in frequency of a single insecticide-resistant clone in the population and its spread across different locations. Thus, Fu's F_s index was negative ($F_s = -25.41$, $p = 0$),

which is a signature of population expansions. In addition, the mismatch analyses showed a unimodal distribution and failed to reject departure from the expansion models, resulting in a nonsignificant Harpending's raggedness index (0.0001, p = 1) for the demographic and spatial expansion models, and nonsignificant sum of squared deviation (SSD) for the spatial expansion model (p = 0.438) while significant at the 5% for the population expansion model (p = 0.031).

5.5. Discussion

The results from the present study provide information about the evolution of two closely related species of cereal aphids under different environments and agricultural landscapes. The study demonstrates that the populations of *S. miscanthi* and *S. avenae* in China and England have evolved in the last 5 to 20 years, which could be as a result of environmental and human-induced changes such as insecticide use, and the genetic structure and diversity have changed in comparison with that observed in earlier studies. This contrasts with what it has been observed previously in another cereal aphid in England, *Rhopalosiphum padi*, whose population has not shown any change in genetic diversity or structure at least since 2003 (Morales-Hojas et al., 2019).

S. miscanthi in China has a higher diversity than previously identified (Guo et al., 2005; Wang et al., 2016). This can be explained by the higher number of genome-wide molecular markers that have been used in the present analysis in comparison with the five microsatellites of previous studies, but the estimated levels of genetic differentiation were similar to those observed in *S. avenae* in China using the same five microsatellites (Xin, Shang, Desneux, & Gao, 2014). However, *S. avenae* is known to be present only in Yili, Xinjiang region in the northwest (Zhang, 1999), and the two *Sitobion* species are morphologically very similar (Choe, Lee, & Lee, 2006; Hales, Foottit, & Maw, 2010), so it is likely that samples from China have been identified as *S. avenae* in some studies and as *S. miscanthi* in others (Blackman & Eastop, 2017). In addition, it could also be the case that there is undescribed taxonomic diversity within *S. miscanthi* in China, and the high levels of genetic differentiation observed could be explained by unidentified races. This is the case in Australia, where there are at least three chromosomal races (Hales, Chapman, Lardner, Cowen, & Turak, 1990), so it is possible that the situation in China is complex as well. Indeed, the high genetic differentiation observed in the present analysis between the six genetic clusters identified suggests that there is at least the same number of different taxonomic units, though the present study is not capable to determine whether they represent subspecies, host races, or chromosomal races as in Australia and New Zealand. Also, it is interesting to note that the individuals show little genetic admixture except for samples from Langfang, one sample from Suzhou and one from Pingliang, which have a proportion of SNPs from the GC2 and GC6. This could

be the result of a lack of reproduction between genetic clusters, despite individuals from different clusters coexisting in the same geographic locations.

While *S. miscanthi* in Australia and New Zealand is predominantly functional parthenogenetic (Sunnucks et al., 1996; Wilson et al., 1999), the present study indicates that the populations in China show either a heterozygote deficiency (significant, positive F_{IS}) or are in HWE (not significant F_{IS}), suggesting that the species reproduces predominantly by cyclical parthenogenesis. The significant deficiency of heterozygosity in populations can be explained by the presence of different genetic clusters (Wahlund effect) observed in several populations, and the F_{IS} is not significant for the genetic clusters indicating that they are in HWE (with the exception of the GC2). This contrasts with previous studies that inferred an excess of heterozygotes in the northern populations, characteristic of anholocyclic lineages, while in the southern populations there was a deficiency of heterozygosity, which is observed in inbred sexual populations or the result of admixed populations (Wahlund effect) (Wang et al., 2016). However, it is usually the case that in colder regions populations are cyclical parthenogenetic as the aphids undergo a phase of sexual reproduction to produce eggs to overwinter; on the other hand, southern populations in warmer regimes can survive as parthenogenetic individuals throughout the year. The results of Wang et al. (2016) indicate that the contrary would be the case in Chinese *S. miscanthi*, which is unexpected. Another previous study of S. miscanthi in China showed that northern populations had heterozygote deficiency while the southern populations were in HWE or had an excess of heterozygotes, although the significance was not tested (Guo et al., 2005). The contrasting results between the present and previous analyses are also at the population subdivision. Thus, we observe no significant north–south differentiation at the QHL traditional geographic division of the country, identified in one previous study (Wang et al., 2016), and there is no evidence for isolation by distance (Guo et al., 2005). The population structure identified in the present study and the phylogenetic analysis suggests that there are six highly differentiated genetic clusters, and admixture results suggest that there is little genetic exchange between them. These clusters do not correspond to geographic regions, as observed also in the phylogeny, and individuals from populations geographically separated by long distances can belong to the same genetic cluster. GC1 comprising individuals from only Kunming and GC3 with individuals only from Wuhan are geographically restricted to one location; the Kunming population is more geographically distant to the other locations, which could explain its genetic isolation, although one individual sampled in Kunming shows a GC2 genotype. This suggests that there is long-distance dispersal of *S. miscanthi* aphids across China, although the high differentiation observed between the genetic clusters and the low genetic admixture in individuals suggest low interbreeding between them.

In England, the level of genetic differentiation is low across the different populations of *S. avenae* sampled and there is no evidence for genetic structure.

This is in accordance with what it was previously observed (Llewellyn et al., 2003). This population homogeneity was taken to be the result of long-distance dispersal of aphids, and results from the present study corroborate this. The population of *S. avenae* in England, however, has evolved in the last 15 years. While most of the markers and populations studied in 1997–1998 were in HWE, and the population showed an increase in cyclical parthenogenetic proportion with latitude (Llewellyn et al., 2003), this study shows that the present English population has an excess of heterozygotes and indicates strong clonality. This suggests that anholocycly is predominant across its range, and there is no evidence for cyclical parthenogenesis occurring toward the north of the country as expected. This change in the *S. avenae* population could be the result of insecticide resistance evolution. In 2011, a knockdown resistance (*kdr*) mutation to pyrethroids was detected in England's population of *S. avenae*, and studies showed that the clone that gained this mutation spread and increased its proportion in the population from 2009 to 2014, and was also observed in Ireland from 2013 (Foster et al., 2014; Malloch et al., 2014, 2016; Dewar & Foster, 2017; Walsh et al., 2019). It is therefore likely that this pyrethroid-resistant clone, which is a facultative parthenogenetic clone (Walsh et al., 2019), has continued to spread and increase in proportion, being now dominant in the English population. The single genetic cluster identified by the Bayesian and AMOVA analyses in this study could correspond to this clone. This is supported by the phylogenetic analysis, which shows a topology that can be explained by asexuality of the *S. avenae* population. Also, the levels of gene diversity (as measured by the H_e) are now lower than those observed in 2003 in the panel of four microsatellites (Llewellyn et al., 2003). In addition, analyses of the demographic history of the population in England indicate that there has been a population demographic and spatial expansion. Thus, as one clone gained the resistance to pyrethroids in a given location, it increased in number in the location but also expanded its distribution as it spread to other regions via migration. However, further analyses will need to be carried out to demonstrate this.

Overall, this study shows that the populations of two species of cereal aphids of the genus *Sitobion* have evolved in recent years in two geographically distant regions under different environmental and human-influenced conditions. The diversity of *S. miscanthi* in China needs to be investigated more comprehensively, as the high level of genetic differentiation suggests the existence of yet unidentified forms. In contrast, the diversity of *S. avenae* has been affected by the evolution of pyrethroid resistance as shown in previous studies, and a single genetic cluster is now dominating the English population as shown in this present study. Although it is possible that the identified genetic cluster corresponds to the insecticide resistance clone, further analyses are needed to demonstrate this. In contrast to this, *S. miscanthi* has not gained insecticide resistance despite having been subject also to its use. In England, the bird cherry—oat aphid, *R. padi*, has not evolved resistance to insecticides either, despite being sympatric with *S. avenae* and therefore subject to the same agricultural practices. Why some species

evolve resistance while other do not it is still a matter of study. It has been shown in *Drosophila melanogaster* that thermotolerance influences the development and spread of insecticide resistance (Fournier-Level et al., 2019). Similarly, the distribution of cyclical and obligate parthenogenetic aphids is strongly influenced by temperature, so it is possible that there is an indirect relationship between life-cycle type and insecticide resistance evolution in aphids. Further studies in aphids would need to be carried out to test whether there is a relationship between thermotolerance, life-cycle types, and the evolution of the *kdr* and super-*kdr* mutations; for this, the population of *S. avenae* in England, where it is predominantly anholocyclic, its sympatric population *R. padi* and the related species *S. miscanthi* in China, which are predominantly cyclical parthenogenetic, can be a useful system to test the hypothesis.

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Chapter VI: General discussion and perspectives

General conclusion and perspectives

Migration is a behavioral strategy formed by insects in the process of long-term adaptation to environmental changes, it is also the main reason for the large number of insects and the frequent outbreaks of migratory pests (Hu et al., 2018; Knight et al., 2019). Migration is often affected by many environmental factors. Among them, temperature plays an important role in the normal physiological activities of insects, and seriously affects the migration behavior (Dai et al., 2017; Wainwright et al., 2017). Therefore, studying the life history traits of insects at different temperatures and clarifying their adaptation mechanisms can better predict the occurrence of aphids migratory behavior and better serve the integrated pest management (Li et al., 2020; Miao et al., 2020; Shi et al., 2020).

In the second chapter, the comparisons of growth, development, survival and fecundity of six geographic populations of *S. miscanthi* individuals were tested from different main wheat areas of China under different temperature conditions. Life table analysis and correlation analysis between geographic factors and *S. miscanthi*'s longevity were also carried out. The development duration and adult longevity of aphid nymphs of each geographic population gradually decreased with the increase of temperature, except that the Yinchuan population was opposite to the above trend at higher temperature. This result may be explained by the fact that there are migratory behaviors among aphids from different populations. Wheat aphids generally move northward with the southwesterly airflow in March and then move southwardly with the northwesterly wind after August to become a source of insects on autumn seedlings in winter wheat areas in China. Therefore, the sources of aphids collected in the same place may be different, which also leads to uncertainty in the results. The possible interference of migration cannot be ruled out. Therefore, further studies are required to evaluate the impacts of aphid migration.

S. miscanthi exhibits an intermediate temperature preference. The negative effects of low and high temperatures on population parameters have been observed in other species (Castillo et al., 2006). Therefore, the influence of temperature on the control efficiency and population fitness of aphids has been estimated in many previous studies, such as one study examining *Aphidius gifuensis* (McCalla et al., 2019). In future research, we will explore the impacts of extreme thermal temperatures on survival and the population fitness on aphids. There was no significant difference in fecundity among the six populations at colder condition, while there was a significant difference within the southern population when hotter. Fecundity of Suzhou and Kunming was significantly higher than that of Wuhan. There was no significant difference in reproductive capacity within northern populations. The development speed of *Laodelphax striatellus* was linearly related to temperature between 15 and 25 ℃. At 30 or 32.5 ℃, the relationship was no longer linear, and the variation increased (Hachiya, 1990). Temperatures higher than 32 ℃ caused a decrease in the developmental rate of *Aphis spiraecola* (J. J. Wang et al., 2000).

In addition, latitude was positively correlated with aphids' longevity. Geographical factors exhibit a certain correlation with the reproductive ability of aphids at different temperatures. Aphids in cooler regions of high latitudes display larger thermal tolerance ranges and exist below their thermal optima. Climate warming could act to increase fitness and ultimately increase aphid populations and their potential as pests. The fecundity of southern populations was generally higher than that of northern populations. In addition, the microenvironment in which insects live yields temperature heterogeneity (Sinoquet et al., 2007, Kearney et al., 2009). These results help to predict potential aphids' outbreaks in China. Assessing facultative endosymbionts in the different populations better reveal the aphid thermal tolerance (Burke et al., 2009; Ferrari et al., 2020). There are close links between aphid responses to heat and to infection. Further research on these questions would be a useful way of the effect of temperature on aphids.

After studying the influence of a single variable-temperature on the life history traits of *S. miscanthi*, we wanted to further explore the migration pathway of the aphids in China. Six typical geographic populations (Wuhan; Suzhou; Kunming; Langfang; Tai'an; Yinchuan) from wheat regions in China were selected, and the mitochondrial *COI* gene of the aphid was combined with two genes of the primary symbiotic bacteria *B. aphidicola* for PCR amplification, sequencing and population genetic structure analysis. Six geographical populations were divided into three groups of Yinchuan, Suzhou and other populations based on three genetic marker genes. There were significant genetic differences among the three groups. At the same time, the results of genetic structure analysis based on mitochondria and symbiotic genes were mostly consistent, indicating that symbiotic bacteria have potential significance in the genetic structure and diversity of aphid populations research. The neutral test values of Fu's *Fs* and Tajima's *D* were negative, which indicates that the population of the aphid population in China had undergone a significant population expansion process in the past.

Further phylogenetic analysis revealed that there were frequent gene exchanges between Wuhan, Kunming, Tai'an and Langfang. These four populations almost contain all haplotypes. There were also haplotypes in Yinchuan and Suzhou populations that were the same as the other four sites. The populations of Yinchuan and Tai'an contained the same rare haplotypes, and Suzhou and Wuhan also had the same rare haplotypes. So, there was a genetic exchange between the three groups. We therefore speculate that the wheat filling period in the Huang-Huai-Hai wheat region in April and May, and the southeast monsoon prevailed, the Yinchuan population moved into the source of alien aphids. The Suzhou population moved into the source of alien aphids from the wheat region of the Yangtze River with the northwest monsoon in September and October. This is consistent with the conclusion of Dong et al.'s research that Yinchuan has an alien source of aphids and the phenomenon of long-distance migration (Dong et al., 1987). Yang et al. also pointed out that *S. avenae* in Shanxi, Hebei, Shandong, Beijing, Tianjin and other places north of the 0℃ isotherm may have migrated from southern Henan, Jiangsu, northern Anhui (Yang et al., 1991). The group

division of aphid in this experiment is basically consistent with the research results. Compared with the traditional methods of studying insect migration, molecular markers can be used to study the genetic diversity of populations and speculate the migration pathway more conveniently and quickly. The annual average temperatures in Yinchuan and Suzhou were the highest and the lowest among the six regions respectively. Therefore, we hypothesized that the difference in the annual average temperature of the collection site may be the cause of the genetic structure of diversity. In summary, understanding the genetic structure of different geographic populations of aphids may provide theoretical significance for the migration of aphids.

Mitochondrial gene of *S. miscanthi* and the ones from endosymbiont were not completely consistent. Based on this, it can be inferred those multiple types of molecular markers may be more reliable for genetic difference research and reflect the real situation, than more accurate and convincing results can be obtained. Previous studies have found that the rate of synonymous mutations in the *Buchnera* genome is at least twice that in the mitochondrial genome (Liadouze et al., 1996; Douglas, 1997; Sato et al., 1997; A. E. Douglas, 1998; Bernays et al., 2010). Given the history of the symbiotic relationship between aphids and *Buchnera*, it has been suggested that molecular markers in *Buchnera* could be used for genetic research in aphid populations. Our next research expanded the collection of 18 different geographic populations of *S. miscanthi*. The aphid mitochondrial *COI*; nuclear gene *EF-1α*; two primary endosymbionts *Buchnera* genes, *gnd* and *trpA* were combined for PCR amplification, sequencing and population genetic structure analysis. The genetic differentiation among aphid populations was analysed using the two endosymbiont *Buchnera* genes, *gnd* and *trpA*. Most populations' pairwise F_{ST} values based on *COI* and *gnd* were extremely differentiated. Only a few pairs had no genetic differentiation. The results provided strong support for the population diversity analysis based on mitochondrial and nuclear genes. Because of the higher number of polymorphic loci, the identification of symbiotic haplotypes was more refined, allowing a more accurate analysis of haplotypes among the populations. Comprehensive analysis of sequence polymorphism sites and genetic polymorphisms showed that genes from symbiotic bacteria could provide more genetic information for population differentiation studies than mitochondrial or nuclear genes alone.

China is a country with diverse geographical forms. The height of the terrain is distributed in three "steps" from west to east. Wheat is suitable for cultivation in temperate and subtropical climates, so wheat is mostly grown at two altitudes in eastern China (Xu et al., 2015). In terms of precipitation, wheat cultivation is performed in the monsoon zone in eastern China, where sufficient precipitation from the ocean can be obtained. Historically, wheat has been planted in China for more than 8,000 years. Since the Western Han Dynasty, wheat has been widely grown in the middle and lower reaches of the Yangtze River, the Huanghuaihai Basin, and Shandong, as these areas have sufficient water for wheat cultivation.

The life history of *S. miscanthi* shows that it can overwinter south of the 0℃ isotherm in January and survive in the north below 26℃ in July. The perennial

habitat of *S. miscanthi* in China is also located in the Yunnan-Guizhou Plateau, the Sichuan Basin, the middle and lower reaches of the Yangtze River, the Huanghuaihai Basin, and the Jiaodong Peninsula. The study is based on the cluster analysis of the populations, which suggests that central China and the Jiaodong Peninsula are the main sources of *S. miscanthi* in China and that the Yunnan-Guizhou Plateau is a secondary source. The values of nucleotide diversity from Qingdao and Yantai were very high for the four genes. Wuhan and Xinxiang also had high nucleotide diversity value for the *COI* gene. Through cluster analysis of the haplotype sequences of *gnd*, we could see that Qingdao, Tai'an and Yinchuan had unique haplotypes. The network diagram structure formed by the molecular markers of symbiotic bacteria was similar to the network diagram structure formed by mitochondria, and both were two main star-mounted structure network diagrams. This indicated that there may be two major population expansion events in the aphid population in China. Many small insects have also been found to be able to have a long-distance migration by air (Wei et al., 2015), and we could infer that these aphids appear first in the southwest and central regions and spread to the north with the help of the southeast and southwest monsoons in spring and summer. In autumn, they could spread to the south with the northeast and northwest monsoons.

However, the above inference is based on strong host specialization of *S. miscanthi* because it examines the genetic differentiation of 18 geographic populations. However, *S. miscanthi* has multiple hosts, and whether these populations are closely related to *S. miscanthi* populations on other hosts requires further study. In addition, the sample collection in this study was limited by time, some iconic populations (such as that in Tibet) were not collected, and there were certain limitations in estimating the aphid migration paths. We look forward to collecting and comparing additional populations of *S. miscanthi* from surrounding regions and conducting follow-up research.

Most *S. miscanthi* populations have high genetic diversity. We infered that Central China and the Jiaodong Peninsula were the main sources of *S. miscanthi* in China, and the Yunnan-Guizhou Plateau was a secondary source. The *S. miscanthi* population in China exhibits diversified reproduction patterns and significant genetic differentiation. The genetic differentiation of the *S. miscanthi* population is closely related to the geographical environment and climate. However, no geographical isolation has been observed.

Finally, we compared the population structure and history of grain aphids in different environments and agricultural landscapes in UK and China.

S. miscanthi is the dominant species of aphids in various wheat regions in China. It has been mistakenly identified as *S. avenae* in China, and it is still widely used (Xin et al., 2019). *S. avenae* is always the dominant species of aphids in UK.

The results from the present study provide information about the evolution of two closely related species of cereal aphids under different environments and agricultural landscapes. The study demonstrates that the populations of *S. miscanthi* and *S. avenae* in China and England have evolved in the last 5 to 20

years, which could be as a result of environmental and human-induced changes such as insecticide use, and the genetic structure and diversity have changed in comparison with that observed in earlier studies. This contrasts with what it has been observed previously in another cereal aphid in England, *R. padi*, whose population has not shown any change in genetic diversity or structure at least since 2003 (Morales-Hojas et al., 2020).

Results of the study indicate that *S. miscanthi* in China has a higher diversity than previously identified (Guo et al., 2005). This can be explained by the higher number of genome-wide molecular markers that have been used in the present analysis in comparison with the five microsatellites of previous studies, but the estimated levels of genetic differentiation were similar to those observed in *S. avenae* in China using the same five microsatellites (Xin et al., 2019). However, *S. avenae* is known to be present only in Yili, Xinjiang region in the northwest (Zhang, 1999), and the two *Sitobion* species are morphologically very similar (Hales et al., 2010), so it is likely that samples from China have been identified as *S. avenae* in some studies and as *S. miscanthi* in others. In addition, it could also be the case that there is undescribed taxonomic diversity within *S. miscanthi* in China, and the high levels of genetic differentiation observed could be explained by unidentified races.

The present study indicates that the populations in China show either a heterozygote deficiency (significant, positive F_{IS}) or are in HWE (not significant F_{IS}), suggesting that the species reproduces predominantly by cyclical parthenogenesis. The significant deficiency of heterozygosity in populations can be explained by the presence of different genetic clusters observed in several populations, and the F_{IS} is not significant for the genetic clusters indicating that they are in HWE (with the exception of the GC2). This contrasts with previous studies that inferred an excess of heterozygotes in the northern populations, characteristic of anholocyclic lineages, while in the southern populations there was a deficiency of heterozygosity, which is observed in inbred sexual populations or the result of admixed populations (Wahlund effect) (Y. Wang et al., 2016). However, it is usually the case that in colder regions populations are cyclical parthenogenetic as the aphids undergo a phase of sexual reproduction to produce eggs to overwinter; on the other hand, southern populations in warmer regimes can survive as parthenogenetic individuals throughout the year. The results indicate that the contrary would be the case in Chinese *S. miscanthi*, which is unexpected. Another previous study of *S. miscanthi* in China showed that northern populations had heterozygote deficiency while the southern populations were in HWE or had an excess of heterozygotes, although the significance was not tested (Guo et al., 2005). The contrasting results between the present and previous analyses are also at the population subdivision. Thus, we observe no significant north–south differentiation at the QHL traditional geographic division of the country, identified in one previous study (Y. Wang et al., 2016), and there is no evidence for isolation by distance (Guo et al., 2005). The population structure identified in the present study and the phylogenetic analysis suggests that there are six highly differentiated genetic clusters, and admixture results

suggest that there is little genetic exchange between them. These clusters do not correspond to geographic regions, as observed also in the phylogeny, and individuals from populations geographically separated by long distances can belong to the same genetic cluster. GC1 comprising individuals from only Kunming and GC3 with individuals only from Wuhan are geographically restricted to one location; the Kunming population is more geographically distant to the other locations, which could explain its genetic isolation, although one individual sampled in Kunming shows a GC2 genotype. This suggests that there is long-distance dispersal of S. miscanthi aphids across China, although the high differentiation observed between the genetic clusters and the low genetic admixture in individuals suggest low interbreeding between them.

Overall, this study shows that the populations of two species of cereal aphids of the genus *Sitobion* have evolved in recent years in two geographically distant regions under different environmental and human-influenced conditions. The diversity of *S. miscanthi* in China needs to be investigated more comprehensively, as the high level of genetic differentiation suggests the existence of yet unidentified forms. In contrast, the diversity of *S. avenae* has been affected by the evolution of pyrethroid resistance as shown in previous studies, and a single genetic cluster is now dominating the English population as shown in this present study. Although it is possible that the identified genetic cluster corresponds to the insecticide resistance clone, further analyses are needed to demonstrate this. In contrast to this, *S. miscanthi* has not gained insecticide resistance despite having been subject also to its use. In England, the bird cherry-oat aphid, *R. padi*, has not evolved resistance to insecticides either, despite being sympatric with *S. avenae* and therefore subject to the same agricultural practices. Why some species evolve resistance while other do not it is still a matter of study. It has been shown in *Drosophila melanogaster* that thermotolerance influences the development and spread of insecticide resistance (Mutero et al., 1994). Similarly, the distribution of cyclical and obligate parthenogenetic aphids is strongly influenced by temperature, so it is possible that there is an indirect relationship between life-cycle type and insecticide resistance evolution in aphids. Further studies in aphids would need to be carried out to test whether there is a relationship between thermotolerance, life-cycle types, and the evolution of the *kdr* and super-*kdr* mutations; for this, the population of *S. avenae* in England, where it is predominantly anholocyclic, its sympatri population *R. padi* and the related species *S. miscanthi* in China, which are predominantly cyclical parthenogenetic, can be a useful system to test the hypothesis.

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Appendix – Publications

1. **Sun, J**., Li, Q., Tan, X., Fan, J., Zhang, Y., Qing, Y. , Francis, F., Chen, J. 2020. Population genetic structure of *Sitobion miscanthi* in China. *Journal of integrative agriculture*, 19, 2-11.

2. **Sun, J**., Li, Q., Francis, F., Chen, J. 2021. Analysis of genetic structure of *Sitobion miscanthi* (Takahashi) from six geographic populations in China based on mitochondrial and primary symbiotic gene. *Communications in Agricultural and Applied Biological Sciences*.

3. **Sun, J**., Tan, X., Li, Q., Francis, F., Chen, J. Effects of different temperatures on the development and reproduction of *Sitobion miscanthi* from six different regions in China. *Frontiers in Ecology and Evolution* (submitted).

4. Morales‐Hojas, R., **Sun, J**., Li, Q., Iraizoz, F.A., Tan, X., & Chen, J. 2020. Contrasting population structure and demographic history of cereal aphids in different environmental and agricultural landscapes. *Ecology and evolution*, 10, 9647-9662.