Analysis of the evolutionary relationship and geographical patterns of genetically varied populations of diamondback moth, *Plutella xylostella* (L.)

by

Shijun You

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Abstract

The diamondback moth (DBM), *Plutella xylostella*, is well known for its extensive adaptation and distribution, high level of genetic variation and polymorphism, and strong resistance to a broad range of synthetic insecticides. Although understanding of the *P. xylostella* biology and ecology has been considerably improved, knowledge on the genetic basis of these traits remains surprisingly limited. Based on data generated by different sets of molecular markers, we uncovered the history of evolutionary origin and regional dispersal, identified the patterns of genetic diversity and variation, characterized the demographic history, and revealed natural and human-aided factors that are potentially responsible for contemporary distribution of *P. xylostella*. These findings rewrite our understanding of this exceptional system, revealing that South America might be a potential origin of *P. xylostella*, and recently colonized across most parts of the world resulting possibly from intensified human activities. With the data from selected continents, we demonstrated signatures of localized selection associated with environmental adaptation and insecticide resistance of *P. xylostella*. This work brings us to a better understanding of the regional movement and genetic bases on rapid adaptation and development of agrochemical resistance, and provides a solid foundation for better monitoring and management of this worldwide herbivore and forecast of regional pest status of *P. xylostella*, by taking a cost-effective response to insecticide resistance and better implementation of biological control programs.

Lay Summary

The diamondback moth, *Plutella xylostella*, is a notorious and globally distributed lepidopteran pest of cruciferous vegetables with extensive adaptation and strong resistance to a broad range of synthetic insecticides. Aiming at better understanding the underlying mechanisms that are responsible for rapid development of resistance to agrochemicals, we investigated the genetic diversity, variation and differentiation of diamondback moth populations in various parts of the world (East Asia and the Americas), by considering the evolutionary relationships and demographic history of the sampled populations. By using different sets of molecular markers, the genetic polymorphism, evolutionary origin, as well as regional patterns of dispersal of diamondback moth were revealed in our target continents. These findings enrich our knowledge about the regional movement and genetic bases on rapid adaptation and development of agrochemical resistance, and provide a solid foundation for better monitoring and management of this worldwide herbivore and forecast of regional pest status.

Preface

Chapter 1 introduces the thesis framework, literature review and research objectives, while Chapter 5 provides the novel findings and potential directions for future research.

For Chapter 2, Shijun You, Fushi Ke, and Dr. Carl Douglas identified the research questions. Shijun You, Fushi Ke, Dr. Liette Vasseur, Dr. Minsheng You and Dr. Carl Douglas designed the research experiments. Shijun You, Fushi Ke, Tiansheng Liu, and Dr. Weiyi He performed the experiments and carried out the data analysis. A version of Chapter 2 has been published (Ke et al, 2013). Shijun You and Fushi Ke wrote most of the manuscript, and all authors contributed to writing the manuscript.

For Chapters 3 & 4, Shijun You, Fushi Ke, Dr. Liette Vasseur, Dr. Geff Gurr, Dr. Minsheng You, and Dr. Carl Douglas identified the research questions and designed the research experiments. Shijun You and Fushi Ke conducted all the research and data analysis. Shijun You was responsible for the text writing.

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List of Abbreviations

DBM	Diamondback moth
Bt	*Bacillus thuringiensis*
IPM	Integrated pest management
RFLP	Restriction fragment length polymorphisms
AFLP	Amplified fragment length polymorphisms
SSR	Simple sequence repeats
mtDNA	Mitochondrial DNA
AGE	Agarose gel electrophoresis
AMOVA	Analyses of molecular variance
HWE	Hardy-Weinberg equilibrium
SNPs	Single nucleotide polymorphisms
COI	Cytochrome c oxidase I
Cytb	Cytochrome b
NadhI	NADH dehydrogenase subunit I
NJ	Neighbor-joining
ML	Maximum likelihood
AIC	Akaike Information Criterion
TMRCA	Time to the most recent common ancestor
DDT	Dichlorodiphenyltrichloroethane
LD	Linkage disequilibrium
MAF	Minor allele frequency
PSMC	Pairwise sequential Markovian coalescence
NA	North America
SA	South America
ABC	ATP-binding cassette
GSTs	Glutathione S-transferases
COEs	Carboxylesterases

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Chapter 1 Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is considered to be the most destructive and globally distributed lepidopteran agricultural pest of *Brassica* vegetables (Talekar and Shelton, 1993; Sarfraz et al, 2005). It was recently estimated that this pest causes a total of 4-5 billion dollars associated with damage and management worldwide per year (Zaluchi, 2012; Furlong, et al., 2013). The absence of effective natural enemies and broad resistance to various insecticides are thought to be the principal causes for frequent outbreaks of *P. xylostella* in many parts of the world (Lim, 1986; Talekar and Shelton, 1993; Li et al., 2016). With the conspicuous features of broad distribution, rapid development of insecticide resistance, and a hostplant range including many economically important food crops such as rapeseed, cauliflower and cabbage, *P. xylostella* has been receiving a great deal of scientific and public attention. This is well reflected by the organization of the Working Group on Diamondback Moth, a regular series of international workshops on its biology and management since 1985, and a large body of research publications with three reviews published in the top entomology journal, the *Annual Review of Entomology* (Talekar and Shelton, 1993; Furlong et al., 2013; Li et al., 2016).

1.1 Biology and ecology

Life history

The practical importance of *P. xylostella* is clear by its relatively short life cycle potentially producing many generations a year, varying and mainly determined by temperature (Li et al., 2016). *P. xylostella* can develop and reproduce over a broad range of temperatures, between 8 - 33℃, with the highest survival and fecundity at 25℃ (Dan, 1995). The annual number of generations per year tends to increase from north to south, with 2 - 4 generations in northeast China and the northern United States (Zhou et al., 2013; Philips et al., 2014), and more than 20 generations in tropical regions where crucifers are grown throughout the year (Talekar and Shelton, 1993; Lin et al., 2013).

P. xylostella adults become active at dusk, when most mating and oviposition occurs (Harcourt, 1957). Egg development varies with temperature, ranging from 2 to 20 days (Harcourt, 1957; Liu

et al., 2002). Damage to hosts is exclusively produced by larval feeding. The larval stage of *P. xylostella* includes four instars and generally requires 2 - 4 weeks to complete (Harcourt, 1957; Liu et al., 2002). When the fourth instar completes feeding, it constructs a loose silken cocoon on the leaf surface where it spends a two day period of quiescence before entering into the formal pupal stage. The duration of the pupal period is temperature-dependent as well, ranging from 5 to 15 days (Harcourt, 1957; Hoy, 1988).

P. xylostella has a high reproductive potential, which is one of the factors making it difficult to control. Female adults start laying eggs soon after mating, and oviposition lasts for a period of 4 - 12 days with a single female depositing up to 350 eggs with an average of 150 eggs (Harcourt, 1957). The optimal temperature for oviposition, with the peak number of eggs laid, ranges from 20 - 25℃ (Liu et al. 2002). Oviposition was observed to mostly occur at night, and is correlated with light intensity and the time of illumination (Harcourt, 1966; Ke and Fang, 1980). Adults are able to feed on nectar as their supplementary food after eclosion. Both life-span and fecundity of adults are correlated with nutritional quality (Ke and Fang, 1980; Talekar and Shelton, 1993).

Natural enemies

A total of 90 species of parasitoids have been documented for *P. xylostella*, with hymenopterans most commonly observed in fields by attacking larvae (Goodwin, 1979; Philips et al., 2014). The most predominant larval parasitoids are from the genera *Diadegma* and *Cotesia* (Lim 1986). In South America, *Diadegma insulare*, *D. leontiniae*, and *Apanteles piceotrichosus* are the dominant species; while *Diadegma insulare*, *Microplites plutellae*, and *Oomyzus sokolowskii* are most frequently found with high parasitism rates in North America. Across farmlands in Asia, *Cotesia vestalis*, *Diadegma semiclausum*, and *O. sokolowksii* are the most effective larval parasitoids.

Arthropod predators, including vespids, syrphids, anthocorids, and spiders, are thought to cause high larval mortality of *P. xylostella*, however owing to a lack of evidence from experimental studies, field efficacy of arthropod predators against *P. xylostella* remains poorly unknown (Suenaga and Hamamura 1998; Furlong et al, 2013). Little effort has been placed on investigating contributions of endemic predators in suppressing *P. xylostella* populations, and the

use of commercial predators in integrated pest management programs lacks promise in the near term.

Various entomopathogens, including viruses, fungi, and nematodes, have presented desirable insecticidal effects in laboratory studies (Furlong et al., 2013). Although a few entomopathogenic viruses and fungi have been commercialized, *Bacillus thuringiensis* (*Bt*) remains the only widely adopted agent useful against *P. xylostella* infestation (Philips et al., 2014). As the first insect species to develop field resistance to *Bt* toxins, further work is required to confirm the practical role of this microbial agent for integrated pest management (IPM) of *P. xylostella*.

DBM-Host plant interactions

Glucosinolates are plant secondary compounds commonly occurring in cruciferous plants and they can be hydrolyzed to volatile isothiocyanates by the endogenous plant enzyme, myrosinase (Renwick 2002). Volatile isothiocyanates are semiochemicals that stimulates *P. xylostella* oviposition (Renwick et al., 2006). Therefore, physiological conditions of host plants, such as activity of myrosinase and release of volatile substances, affect the odor reception of *P. xylostella* adults and subsequent oviposition activity (Furlong et al., 2013).

Larval feeding of *P. xylostella* induces changes in glucosinolate (Girling, et al., 2011; Textor and Gershenzon, 2009) and volatile profiles (Girling, et al., 2011; Kugimiya et al., 2010) of host plants. Parasitoids, such as *Cotesia vestalis* and *D. semiclausum* are attracted to volatiles emitted from *P. xylostella*-infested host plants (Bukovinszky et al., 2005; Potting et al., 1999). However, performance and fitness of DBM (Sarfraz et al., 2007; Soufbaf et al., 2010) and its parasitoids are affected by multiple complex factors, such as nutritional status of host plants, fertilizers, and composition of feeding-induced volatile blends, and there is still a knowledge gap about *P. xylostella*-parasitoid population dynamics as mediated by host plants (Karimzadeh et al., 2004).

1.2 Pest status and management

The ancestral origin of *P. xylostella* remains controversial. Hardy et al. (1938) first proposed that DBM originated from the Mediterranean region, and spread over all continents with the

distribution of crucifers by humans. This proposed origin of DBM was broadly acknowledged until other evidence became available in the 1990s. Based on documentation of obligate parasitic wasps on larvae and pupae of *P. xylostella* in South Africa, Kfir (1998) believes that there should be a long history of the linkage between these parasitic wasps and *P. xylostella* in that area, suggesting that DBM originated in Africa. Liu et al. (2000) proposed that the origin of *P. xylostella* was in China, based on its parasitic natural enemies, the large number of native cruciferous vegetables, as well as the long history of crucifers cultivation in the country. A recent study (Juric et al., 2016) supports the claim of Africa as the most probable origin, but cannot preclude Asia as an alternative based on the genetic structure of *P. xylostella* populations sampled in 16 geographical locations, with one sample from Africa, 11 from Eurasia, 2 from North America, 2 from Oceania but without samples from South America. All of these speculations with respect to the origin of *P. xylostella* are inferential hypotheses that have not yet been tested with geographically sufficient data worldwide and convincing analytical approaches.

Although there was an atypical observation of DBM populations on pea, *Pisum sativum* in 1999, Brassicaceae is the only widely accepted host plant family for *P. xylostella*, with extreme preference for mustard oil glycosides (= glucosinolates) produced by cruciferous plants (Furlong, et al., 2013). Worldwide, there are more than 40 cruciferous vegetable species of considerable economic importance documented as the most common host plants for *P. xylostella*; it is also universally believed that brassicaceous weeds, as alternative hosts, are of great importance in maintaining DBM populations (Talekar and Shelton, 1993; Furlong et al., 2013). Impacts of *P. xylostella* had been observed in at least 84 countries/regions by the 1930s (Hardy, 1938; Ke and Fang, 1980); while damage by *P. xylostella* had been documented in approximately 120 countries/regions by 1972 (Lim, 1986). After the 1980s, observation of *P. xylostella* has been reported in all crucifer-growing regions worldwide (Sarfraz et al, 2005). However, *P. xylostella* was not a key pest of crucifers prior to the 1930s (Lim, 1986), and infestations rose increasingly with the popularity of insecticides beginning in the late 1940s around the world, bringing devastating destruction of cruciferous crops. Asian countries, including China, Japan, Malaysia, Thailand, and Philippines, have since suffered from moth outbreaks with up to 90% yield losses in vegetable production (Verkerk and Wright, 1996). Pakistani farmers even gave up vegetable planting during a devastating moth infestation (Abro et al, 1994). *P. xylostella*, is a major

agricultural pest in the southeastern US and Pacific States, and affects all states in the USA (Brown et al, 1999). It is the critical foliar pest affecting American canola production (Ramachandran et al, 2000). *P. xylostella* was introduced to Canada in the 1880s, and now causes year-round damage to brassicaceous crops (Dosdall et al. 2004; Lee, 2013). As one of the most difficult pest insects to control, the estimated annual cost for global *P. xylostella* management and its associated losses reaches 4-5 billion US dollars (Sarfraz et al, 2005; Furlong et al., 2013).

Various approaches, mainly relying on application of agrochemicals, have been frequently employed to reduce infestations of *P. xylostella*. Broad and heavy use of insecticides creates long-term exposure, generating selective pressure for resistant *P. xylostella* strains. This results in varying degrees of resistance to almost all applied insecticides, including organophosphate, organochlorine, carbamate, and pyrethroid insecticides, as well as to insect growth regulators (IGR) and microbial insecticides such as Bt (Furlong et al., 2013). In 1953, Ankersmit first reported the resistance of *P. xylostella* to DDT and toxaphene on Java Island, Indonesia (Ankersmit, 1953). Since then, *P. xylostella* resistance has been widely documented in numerous countries and regions (Talekar and Shelton, 1993; Philips et al., 2014; Li et al., 2016). Tabashnik et al. (1990) was the first to observe resistance of *P. xylostella* to *Bt* toxins in 1990. This resistance issue triggers increasing concern towards integrated management of *P. xylostella*.

1.3 Overwintering and migration

Migration of *P. xylostella* is often taken to be associated with its overwintering. Host plant availability and temperature requirements are both met for moth development in tropical and most subtropical zones, in which overwintering diapause of DBM has rarely been reported (Gu, 2009; Ma et al., 2010). For northern temperate zones, it is generally accepted that overwintering ability is limited, although some field and lab observations suggest overwintering capability of adults or pupae by hiding in host plant residues or other warmer refugia (Hardy, 1938; Lu and Chen, 1986; Dosdall, 2004). In recent decades, however, DBM outbreaks and consequent vegetable/crop yield reductions have been widely observed across some of the northern temperate zones in which DBM is believed to be incapable of overwintering, and mass migrations from warmer areas aided by air advection are the most likely explanation based on

the following observations: a) the majority of DBM populations are not able to overwinter in western and central Canada with rare occurrence of extremely small populations (during warm winters), and the annual infestation is predicted to result from external migration from the southern USA or Mexico (Dosdall, 2003); b) *P. xylostella* is not able to overwinter in northern Japan (including Hokkaido, Tohoku, as well as Hokuriku districts of Honshu) with over 2 months of continuous snow cover, and moth populations are likely to be introduced from warmer southwestern areas or subtropical islands (Honda et al., 1992; Saito et al., 1998); c) year-round presence of DBM was documented in the southern USA, e.g., Arizona, New Mexico, and Texas; and consensus regarding moth outbreaks in the northern USA (e.g., Massachusetts, New York, Minnesota, Wisconsin) is that such infestations primarily arise from migration and transportation of vegetable seedlings from the south, although occasionally plant debris provides temporary shelters for moth hibernation (Andaloro, 1983; Idris and Grafius, 1996); and d) Ma et al. (2010) described the inability of DBM to overwinter in northeastern China, where moth populations are introduced by southwest air currents. DBM capable of hibernation are able to pass their insecticide resistance to future generations, while the localized resistance disappears once populations unable to hibernate are eliminated. In regions without largely observable moth hibernation, therefore, intensified monitoring of mass migration in spring is necessary to minimize potential crop losses.

Infestation of *P. xylostella* is often attributed to its extraordinary migratory capacity, which provides opportunities for extensive gene flow amongst populations. Several investigations, conducted in various regions have demonstrated that *P. xylostella* is able to move over 1000km/day for consecutive days with the assistance of strong air flow (Capriol and Tabashnik, 1992; Chapman et al., 2002). Chu (1986) reported a novel capture of *P. xylostella* on the Pacific Ocean, 500 km from the nearest land. Outbreaks of *P. xylostella* occur yearly in the UK, and the immigrants, traveling up to 3000 km, are largely from the Baltic region (Chapman et al., 2002). Shirai recorded the total flight time of over 11 hours from a two-day observation of DBM at 23°C and found that slightly lower temperature seems to favor moth growth and development with greater longevity, larger body size, and longer forewings, all of which facilitate movement and enable DBM to fly long distances (Shirai, 1993a and b, 1995). However, other noteworthy observations have also been recorded, e.g., that migration of *P. xylostella* may be less than one

km during their entire lifetime with sufficient and accessible food resources (Shirai, 1991; Shirai and Nakamura 1994). Tabashnik et al. (1987) found divergent insecticide resistance amongst populations within a geographic range of 5 km, and the potential explanation for such an observation was the lack of massive migration/gene flow.

Migratory behaviors and capacities of parasitoids attacking DBM are believed to be rather weak, relative to such capacities of their hosts. Artificial introduction of exotic bio-control agents therefore has become one of the most common strategies in various integrated management programs for DBM. However, insufficient knowledge of differences between various DBM and parasitoid lineages, especially in terms of genetic makeup and structure, has led to numerous unsuccessful introductions of exotic bio-control agents, often attributable to misidentification or misunderstanding of such an interative system of DBM and its parasitoids.

1.4 Population genetics and phylogeography

The issue of insecticide resistance is drawing increasing concern as a result of insecticide over use and increasing plantings of transgenic plants containing insecticidal genes (Caprio, 1998, 2001). Genetic diversity basically determines the capacity of a targeted population to withstand adverse environmental conditions and stably maintain itself. Low genetic diversity suggests a greater sensitivity and susceptibility to external pressure and changing environment, through the lack of potential alleles for greater fitness under novel conditions (Kirt and Freeland, 2011). Repeated application of insecticides generates selective pressures (reduced reproductive success) on pest populations; resistance to insecticides is therefore dramatically subject to population genetic diversity. Populations with lower genetic diversity are less capable of withstanding adversity which can be exacerbated in the absence of adequate gene flow, giving rise to inbreeding depression and decline of evolutionary potential, even leading to population extinction (Kirt and Freeland, 2011). In contrast, genetically diverse populations contain the potential founders of insecticide resistance, which can be a causative factor in insect pest outbreaks. Owing to their high fecundity and notable migration capability, insect populations are dynamic and gene flow therefore plays an important role in variation of population genetic structure. Understanding population genetic diversity, individual/population movement, as well

as subsequent gene flow is of considerable significance for sustainable pest management, especially as insecticide resistance in *P. xylostella* is closely associated with population genetic structure (Endersby et al., 2006, Roux et al., 2007).

Many previous studies have been carried out on moth biology, ecology, and agrochemical resistance; however DBM is still the pest that most seriously imperils cruciferous plants cultivated in many counties and regions (Furlong et al., 2013). Although mass migration seems favorable for homogenizing population structure via gene flow, the impacts and damage of *P. xylostella*, by contrast, are much more significant in tropical zones. Considerable differentiation in susceptibility to agrochemicals, which is likely to be the result of the variance in inter-population genetic diversity of *P. xylostella*, has been reported previously (Mohan and Gujar, 2003). Much recent research has been conducted on small-scale vegetable field ecosystems. Without consideration of other factors, e.g. gene flow, and population genetic variability, the mechanisms of moth infestation cannot be completely understood and achieving sustainable management of DBM seems uncertain. Therefore, DBM is a compelling model organism for studying global and regional phylogeography of migratory insects and characterization of insect population dynamics, genetic diversity, and phylogeographic relationships. Such studies may better address the origin, dispersal and distribution/colonization, and mechanisms of DBM outbreaks in different countries/regions. Establishing the linkage between inter-population genetic variation of *P. xylostella* and its recent colonization patterns should help support sustainable management of DBM in line with local conditions, based on background information of management on sites of origin, by gradually reducing the reliance on agrochemicals in vegetable production.

Phylogeography, as first proposed by Avise (1987), is an interdisciplinary field that studies the evolutionary processes of different lineages at large spatial and temporal scales (Avise, 2009; Hickerson et al., 2010). To date, various markers have been employed in this field, including allozymes, restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR or microsatellites), and mitochondrial DNA (mtDNA), some of which have been applied to DBM.

Based on polymorphic allozyme loci, Caprio & Tabashnik (1992) and Kim et al. (1999) proposed no significant genetic differentiation for *P. xylostella* populations from the Hawaiin archipelago or within South Korea. Applying an analogous approach, i.e. allozyme electrophoresis, Noran and Tang (1996) suggested a notable genetic divergence amongst *P. xylostella* populations from areas with different altitudes (lowland and highland areas) in Malaysia. There are also comparable studies on population structure of *P. xylostella* at larger scales based on genetic structure (in terms of allozyme polymorphism) of *P. xylostella* populations from various geographical regions (13 sites from 9 countries). Pichon et al (2006) demonstrated an increased genetic divergence index (Fst) relative to previous small-scale studies. Due to relatively limited information regarding general population variation and phylogeographical relationships provided by the allozyme markers, however, DNA sequence-based methods are expected to generate more direct and desirable evidence in order to further reveal demography-related issues (Pichon et al., 2006).

1.4.1 MtDNA-based studies

Gene sequence variation can be applied to phylogenetic analyses at different levels and rapidly evolving genes or loci are more desirable for phylogenetic studies on intraspecies or related species (Zhang, 2004). The mtDNA sequence in animal cells comprises a circular double-helix DNA with approximately 15,000 - 17,000 base pairs. Due to a series of factors, such as incomplete DNA repair mechanisms in the cytoplasm and lack of histones, the base pair substitution rate of mtDNA is approximately 10 times higher than nuclear DNA (Haag-Liautard et al.2008; Hickerson et al., 2010). High intra- and inter-specific polymorphisms therefore enable mtDNA to be a molecular marker to investigate speciation, population genealogy, and population genetic structure (Brito and Edwards, 2009; Finn et al., 2006; Schiffer et al., 2007). However, results can be biased since only maternal history is reflected by mitochondrial genome variation (Zhang and Hewitt, 2003).

Using variation in the mitochondrial cytochrome oxidase I gene, Lunt et al (1998) characterized the population genetic dynamics of the European meadow grasshopper (*Chorthippus parallelus*) and deduced that the current distribution of the Nordic and the Balkan populations, which share a common ancestor, were closely correlated with the dispersal of the Balkan population.

Furthermore, outbreaks of numerous rice insect pests in Japan and Korea are correlated with annual mass migrations. Aiming at identifying the origins of migrations and the population structure of the brown plant hopper (*Nilaparvata lugens*) in Asian rice-growing areas, Mun (1999) analyzed the genetic dynamics of 71 individuals collected in 11 field sites across Eastern and Southern Asia (including Korea, Philippines, China, Bangladesh, Malaysia, Vietnam, and Thailand) based on variation in the 850-bp mitochondrial cytochrome oxidase I gene. The hypothesis that the Korean *N. lugens* populations are the immigrants from Chinese *N. lugens* populations was verified based on finding the same haplotype in both locations. In addition, Ma (2012) investigated the phylogeography and migration route of the migratory locust (*Locusta migratoria*) according to polymorphisms in 3 mitochondrial genes in 263 individuals and mitochondrial genomes in 65 individuals sampled from 53 localities worldwide. The results pointed to a potential origin of *Locusta migratoria* and revealed significant genetic divergence amongst different geographic populations even though long-distance migration was commonly observed for this species. For DBM, Kim et al. (2003) proposed an explanation for the contemporary colonization of *P. xylostella* populations in Korea, in light of the polymorphism of mitochondrial COI gene (fragment) sequence: frequent gene flow results in the absence of considerable genetic variation amongst different populations. One analogous study conducted by Li et al (2006) also agreed on the moth demography that long range dispersal combined with high gene flow rate are the major factors responsible for current distribution and population structure of *P. xylostella* in China. This previous work, based on partial mitochondrial genomic information, has implications for addressing adaptive differentiation, but could be improved with higher resolution in terms of more polymorphic loci or complete mitochondrial genome sequences (Ma et al. 2012).

Investigations of phylogeographic relatgionships have also provided evidence of evolutionary interactions of the host-parasitoid system. Althoff and Thompson (1999) compared phylogenies of two host-parasitoid pairs (*Greya subalba* and *Agathis thompsoni*; *G. enchrysa* and *Agathis n. sp*) over wide geographic ranges, finding no correlations in geographic structure. Population structure of another host-parasitoid system, *Andricus kollari* and *Megastigmus stigmatizans*, showed concordance and followed the host-tracking model (Hayward and Stone, 2006). These studies shed light on underlying forces and mechanisms that might be responsible for

determining population structure and modes of co-evolution of closely interactive organisms.

1.4.2 Microsatellite-based studies

Microsatellite markers (also known as Simple Sequence Repeats, SSRs), with a high level of polymorphism and reproducibility in genotyping (Zhang, 2004), are important tools that are able to specifically measure genetic diversity and divergence, at better resolution than previously used markers, e.g. allozymes, RFLP, AFLP, etc. (Brito and Edwards, 2009; Butcher et al., 2004; Shaw et al., 1999). Polymorphisms of microsatellite loci mainly arise from variation of the number of repetitive units and nucleotide substitution (Weber and Wong, 1993). It is also generally accepted that the greater the number of repetitive units within a microsatellite locus the more likely the level of polymorphism and the greater number of alleles. The wide distribution over the entire genome and high repetition rate of microsatellites make them suitable for studies on populations with relatively low genetic variation by not only distinguishing remarkable genetic differentiation, but also by reflecting the likely geographic distribution in recent evolutionary history (Schiffer et a1. 2007). For example, Margaritopoulos (2009) studied the global genetic variation at 6 polymorphic microsatellite loci of the green peach aphid (*Myzus persicae*), and concluded that worldwide, *M. persicae* diverged into three main strains based on the calculation of genetic coefficient differentiation F_{ST}. Also, unsurprisingly, geographical barriers and distribution of host species substantially shaped the genetic differentiation of *M. persicae* suggesting that the globalization of agriculture has had an important impact on pest population dynamics.

The development of microsatellite DNA markers for lepidopteran insects has been impeded by the relatively low frequency and low efficiency of SSR isolation, potentially due to the existence of microsatellite DNA families (with identical or highly similar flanking regions) that are inappropriate for primer design and likely to confound the final results (Zhang, 2004). Owing to the low frequency plus high sequence redundancy, limited compelling findings on microsatellite studies of Lepidoptera, especially *P. xylostella*, have been generated thus far. Butcher et al. (2004) made a preliminary assessment of microsatellite markers and amplified fragment length polymorphism (AFLP) markers for studying genetic variation of *P. xylostella*, and suggested that microsatellites provide a better immediate prospect for population studies. Endersby et al. (2006)

explored the regional genetic differentiation based on the polymorphism of 6 microsatellite loci and concluded no significant divergence for Australian and North Island (New Zealand) populations but notable divergence for samples from other regions, including Kenya, Malaysia, Indonesia, as well as New Zealand (exclusive of North Island). Such an advanced study, however, seems unconvincing with respect to regional patterns, given that it was based on a single population sample from each of the above regions beyond Australia. Meanwhile, the genetic structure produced by Endersby's group needs further verification due to potential bias derived from only 6 microsatellite loci.

Previous investigations of *P. xylostella* population genetics provide somewhat divergent perspectives; for example, Pichon et al. (2006), Endersby et al. (2006) and Roux et al. (2007) did not reach a consensus regarding moth genetic diversity for Australian populations. One additional determinant to improve study validity and reliability is the range and size of specimen collection. To date, regional and international genetic patterns and dynamics of DBM have been mainly explored by sporadic sampling. Without more intensive specimen collection, any extra lab studies seem futile to generate more significant insights enriching our current knowledge and understanding of moth phylogeography. However, a series of preliminary and inspiring outcomes have been generated thus far, indicating potential orientation for future studies. For example, applying more specific and precise molecule markers, e.g., SSRs are expected to generate more enriching answers to the population-related questions for *P. xylostella*, and facilitate a deeper understanding of mechanisms involved in *P. xylostella* infestation.

It is thought that the phylogeographic structure of agricultural pest populations is being gradually altered through human impacts, such as insecticide use. Therefore, investigation of the phylogenetic relationship, evolutionary and colonization history, as well as genetic differentiation of moth populations could contribute to better management strategies by enriching our understanding of the mechanism (especially molecular mechanism) associated with insecticide resistance development and potential outbreaks over wide spatical scales, so as to mitigate pest problems and favor vegetable production.

1.5 Genomic studies and their utility

Since the first genome sequence of an insect species, *Drosophila melanogaster*, was published in 2000 (Adams et al., 2000), genomic studies of insects have been increasing rapidly with an annual rate of 1-2 genomes released between 2000-2005. The first platform of high throughput sequencing emerged in 2005 (Margulies *et al.*， 2005) and after that, more than10 insect genomes have been sequenced every 2-3 years. With the development of sequencing technologies and declining costs associated with sequencing, over 30 insect genomes have been published per year since 2013, reaching a peak of 67 in 2015 (Yin et al., 2016). In 2011, an initiative entitled "5,000 arthropod Genome Initiative" (i5k), was proposed in a letter to *Science* (Robinson et al., 2011), which provided a detailed roadmap for sequencing and analyzing 5000 high-priority arthropods by the i5k community in 2012 (i5K Consortium, 2013). To date, the previously released insect genomes (including 138 species) are mostly centered on the taxa in relation to human health, agricultural production or food security and environmental protection and include 11 lepidopteran species (8%), 67 dipteran species (48.6%), and 29 hymenopteran species (21%) (Yin et al., 2016).

Taking advantage of the genomic data and approaches, some biological and ecological questions of broad interests have been addressed with identification of genes associated with functional traits in agroecosystems. For example, *Bombyx mori* was domesticated from populations of the wild silkworm, *B. mandarina*, and 354 genes involved in pathways of domestication, silk production, digestion, as well as reproduction have been identified (Xia *et al.*,2009). Whole-genome sequencing demonstrated that contemporary global distribution of the monarch butterfly *Danaus plexippus* originated from a migratory population in North America, followed by three independent dispersal events (Zhan *et al.*, 2014). Genomic study of *Plutella xylostella* shed light on co-evolution between this notorious agricultural pest and its host plants, and helped to better understand mechanisms underlying the detoxification of plant defense compounds (You *et al.*, 2013).

1.5.1 Migration

In agricultural landscapes, dynamic migration and spill-over movement of insects often occurs

between different natural habitats as well as across crop and non-crop interface habitats (Brückmann et al., 2010; Blitzer et al., 2012). Exploring migration of insects in complex landscapes may thus allow for better understanding the effects of diversified landscapes on population and community dynamics of arthropods, and conservation of natural enemies for pest control (Holzschuh *et al.,* 2008; Zhao et al., 2013; Costamagna, 2015).

Approaches of molecular biology, population genetics, and genomics have been increasingly applied in research on long-distance migration and dispersal of insects, which is traditionally investigated by radar observation. Studies on genetic divergence and frequency of gene flow of populations provide evidence for the long-distance migration of several insects, such as dragonflies (May, 2013), the monarch butterfly (Lyons et al., 2012, Pierce et al., 2014, Chapman et al., 2015), the true armyworm (Nagoshi et al., 2012), rice planthoppers (Mun et al., 1999); the cotton bollworm (Behere et al., 2014), the Asiatic corn borer (Li et al., 2014), and the diamondback moth (Endersby et al., 2006, Li et al., 2006). mtDNA-based data analyses have revealed phylogeographic patterns and dispersal routes, leading to a conclusion that ancestral *Locusta migratoria* populations are likely divided into Northern and Southern lineages with allopatry and their current distributions (Ma et al., 2012). *Plutella xylostella* populations were found to usually relocate in China through a northward migration, based on genetic information of nucleus markers and mitochondrial genes (Wei et al., 2013). Comparative genomic studies have identified that the monarch butterfly originated from North America, and then migrated to different locations in the world (Zhan et al., 2014).

1.5.2 Insecticide resistance

Overuse and misuse of chemical insecticides have not only brought many negative impacts on natural ecosystems and human health, but also caused over 500 species of insects to develop resistance (Tabashnik *et al.*, 2014). Pest management schemes place strong selection on insecticide resistance genes, and play important roles in determining the genetic structure of these genes both in local populations and over landscapes (Caprio, 2001; Franck and Timm, 2010; Thaler *et al.*, 2008). For example, variation in the genetic structure of *Cydia pomonella* from France, Italy, Armenia, and Chile was determined to be related to the application of insecticides (Franck et al., 2007).

Local adaptation is shaped by heterogeneous selection over landscapes, but the ultimate consequence of evolution is determined by collective effects of selection strength and gene flow (Postma and van Noordwijk, 2005, Savolainen *et al.*, 2007). Spatial or temporal heterogeneity may delay adaptation over landscapes (Caprio, 2001; Kassen, 2002), especially in the context of optimal connectivity between habitat patches (Vacher *et al.*, 2003). Spatial connectivity between crop and non-crop habitats conserves susceptible populations and impedes development of insecticide resistance (Fuentes-Contreras *et al.*, 2014). For example, gene flow between the *Cydia pomonella* populations from managed orchards (with use of insecticides) and unmanaged habitats delays the evolution of insecticide resistance (Basoalto *et al.*, 2010; Fuentes-Contreras *et al.*, 2014; Ricci et al., 2009). Development of insecticide resistance in *Pectinophora gossypiella* populations may be slowed down when the distance between fields of Bt-transgenic cotton and refugia is optimized at 0.75 km (Carrière *et al.*, 2004). To date, however, there is still no consensus on the optimal scale of spatial and temporal design that can effectively prevent evolution of insecticide resistance.

Selection acts exclusively on genes associated with adaptation (Campbell and Bernatchez, 2004). In addition, gene flow is one of the fundamental forces that drives adaptation and coevolution (Crespi, 2000, Edelaar *et al.*, 2008, Edelaar and Bolnick, 2012). Gene flow between habitats subject to insecticide application or with GM crops and non-GM crops can prevent rapid development of insecticide resistance, which is favorable for pest management (Tabashnik, 2008, Tabashnik and Gould, 2012). Non-crop habitats provide important refugia for natural enemies, especially when using insecticides to control pests in the fields (Schmidt *et al.*, 2005), which may improve diversity of natural enemies and parasitism rates in the fields (Bianchi *et al.*, 2006,).

In general, the recent development of novel DNA sequencing technologies has revolutionized and extended entomological research by providing a wealth of genomic data (Storfer et al., 2015). The availability of a large volume of genomic data has not only allowed for genetic characterization of individuals, populations, and species (Xia et al., 2004; You et al., 2013), but also facilitated profound studies on functional genomics to provide novel insights into the ecology and evolution of insects (Zhan et al., 2014; Wallberg et al., 2014). Large sets of various

kinds of molecular data therefore will enrich our insights on dispersal and local adaptation of the destructive pest, *Plutella xylostella*, with global distribution and extremely strong resistance to agrochemicals, possibly enabling better implementation of cost-effective control measures over larger spatial scales.

1.6 Research Objectives

The overarching goal of my doctoral study therefore is to examine the genetic structure of *Plutella xylostella* in Asia and the Americas to better understand the phylogenetic makeup (over time) and geographical genetic structure (in space) of this species, using various genetic markers, *viz*. microsatellites, mitochondria genes, and genome-wide SNPs.
The hypotheses are that:
a) DBM populations from the two sides of the Taiwan Strait are genetically differentiated.
b) The evolutionary interaction of DBM and its domiant parasitoid, *Cotesia Vestalis*, follows the host-tracking model.
c) DBM populations in North America and South America underwent localized adaptation.

To test these hypothesis, the following objectives were developed:
a) assess genetic differentiation and patterns of diversity among populations in relation to geography across the DBM range in East Asia and the Americas;
b) elucidate the phylogeny/genealogy of sampled DBM (and *C. vestalis*) populations;
c) understand the origin, demographic/distribution patterns, and predicted potential migration routes of DBM in Asia and the Americas.

Such a research project cuts across various aspects of molecular ecology (bringing together molecular biology, population genetics/genomics and phylogenetics) both at the theoretical and applied levels and will enrich the interdisciplinary nature of phylogeography. The following scientific questions/issues will be addressed and guide the project:
a) What is the present genetic variability within and among DBM populations in Asia and the Americas? Is there a relationship between genetic and geographical distances of these DBM populations?

b) How did the DBM populations genetically evolve and differentiate from various geographical locations?
c) Where is the geographical center of origin of DBM? What are the contributions of previous expansions (through migration and colonization processes) to the genetic makeup of DBM?

Chapter 2 examines the genetic differences of *P. xylostella* populations from various locations on both sides of the Taiwan Strait and identifies the variables governing the dynamics of gene flow using microsatellite markers. The results reveal that *P. xylostella* populations can be divided into two distinct clusters, which is likely due to annual airflows in this region. A pattern of isolation by distance among local populations within Fujian Province (PR China) was found, and may be related to vegetable transportation.

Chapter 3 addresses the phylogeographical relationships and potential evolutionary interactions between *P. xylostella* and its parasitoid *Cotesia vestalis* in East Asia, using mitochondrial and nuclear markers. The key finding demonstrates that indigenous *C. vestalis* adapted to *P. xylostella* as a new host by ecological sorting, as *P. xylostella* expanded its geographical range into in East Asia where the parasitoid is posited to have originated.

Chapter 4 investigates the history of evolutionary origin and regional distribution in North and South American *P. xylostella* populations, the patterns of genetic diversity and variation, and characterizes the genomic signatures of local adaptation. The results indicate that *P. xylostella* originated in South America, and recently colonized across both American continents, resulting possibly from intensified human activities.

Chapter 2 Genetic differentiation of the regional *Plutella xylostella* populations across the Taiwan Strait based on identification of microsatellite markers

2.1 Introduction

The genetic makeup of populations is important in determining their capacity to withstand adverse environments and, if needed, adapt to new conditions (Vignuzzi et al. 2006; Draghi et al. 2010; Hayden et al. 2011; Verhoeven et al. 2011). Population structure and connectivity as well as genetic diversity all define the level of susceptibility of a population and its adaptive capacity to environmental changes (Freeland 2006; Kremer et al. 2012; Pauls et al. 2013). Gene flow, through dispersal and short- or long-distance migration, plays a role in determining genetic variation and evolution of local populations (Alleaume-Benharira et al. 2006; Kremer et al. 2012; Raymond et al. 2013; Rius and Darling 2014). For insect pests, gene flow can also facilitate population outbreaks and increase the possibility for the spread of insecticide-resistant genes (Herzig 1995; Margaritopoulos et al. 2009). Different factors, such as the types of human activities, air currents, and climate conditions, as well as the presence of geographic barriers, can facilitate or impede dispersal or migration of insect species (Wei et al. 2013; Niu et al. 2014;). For pest management, understanding how environmental and anthropogenic factors influence individual movements and gene flow is essential at both local and regional levels. Analysis of genetic variation within and among pest populations has been a powerful tool to understand the importance of dispersal or migration and remains an important issue to consider when developing sustainable pest management (Roderick 1996; Raymond et al. 2013).

The diamondback moth (DBM), *Plutella xylostella* (L), represents a typical pest insect that has the capacity to disperse or migrate over short to long distances (Furlong et al. 2013; Philips et al. 2014). This pest of brassicaceous species has been successful in adapting to various environmental conditions and has a worldwide distribution (Furlong et al. 2013). Long-distance dispersal of DBM has been documented and is especially triggered by airflow during favourable meteorological conditions (Chapman et al. 2002; Coulson et al. 2002; Fu et al. 2014). The dynamics of DBM movement at local and

regional scales, however, remains less understood and has been suggested to be confined primarily to movement between neighboring fields (Mo et al. 2003; Schellhorn et al. 2008).

Population genetic studies of DBM have been carried out, but few examined explicitly the factors influencing regional genetic distribution (Endersby et al. 2006; Li et al. 2006). Wei et al. (2013) report an overall lack of genetic differentiation among all 27 populations analyzed in China, with no correlation between genetic and geographic distances. The annual migration of DBM from southern to northern regions of China may result from strong winds (Fu et al. 2014) and/ or meteorological events (Wei et al. 2013). At the landscape scale, Niu et al. (2014) argue that mountains can shape the genetic structure of DBM populations and vegetable transportation may be responsible for gene flow among local populations. Tabashnik et al. (1987) report significant intra-island variation in susceptibility to different insecticides among DBM populations of Hawaii and suggest that local factors, such as spraying of conventional insecticides, are probably playing an important role in shaping the genetic structure of DBM populations. These studies suggest that many factors may interact in structuring DBM population genetics. Examining how dispersal or movement mechanisms govern genetic structure and gene flow of this pest can help better understand its ability to rapidly adapt to novel environments.

Fujian and Taiwan are on both sides of the Taiwan Strait where vegetable production, including cruciferous plants, is currently intensifying. Both provinces suffer from frequent infestations of *P. xylostella* (Talekar and Shelton 1993; You and Wei 2007). The Taiwan Strait (averaging 200 km in width) is a natural barrier to dispersal of many species (Ge et al. 2012, 2015; Liu et al. 2013). However, it may not be a movement barrier to this herbivore, as it is known to travel a distance of approximately 400-500 km per night (Chapman et al. 2002). Restrictions in vegetable transportation and trade between Fujian and Taiwan may have limited the movement of the species between the two regions. The year-round monsoons prevailing across the Taiwan Strait with important changes of air current directions over the year may also influence gene flow within and

among populations of this pest. The objectives of the present study were therefore to: (1) examine the genetic differences of *P. xylostella* populations from various locations of both sides of the Taiwan Strait and (2) identify the variables governing the dynamics of gene flow and the *P. xylostella* population genetic structure using microsatellite markers.

I used selectively neutral molecular markers to study genetic differentiation in DBM, as they are preferred for studying questions of demographic history as well as gene flow (Cooke and Lees 2004; Meng et al. 2015). From a landscape genetic viewpoint, neutral molecular markers such as simple sequence repeats (SSRs) are optimal in estimating population parameters, because they can give unbiased estimation of genetic diversity, migration rates, and population structure (Manel et al. 2003; Schwartz et al. 2010). High polymorphism and co-dominance make SSRs suitable for studying populations by not only distinguishing remarkable genetic differentiation, but also providing insights into fine-scale ecological entities (Roderick 1996; Sunnucks 2000; Selkoe and Toonen 2006). I first isolated effective and neutrally-inherited SSR (or microsatellite) markers from the *P. xylostella* transcriptome and then used the polymorphic loci for the genetic analysis of *P. xylostella* populations collected from both sides of the Taiwan Strait.

2.2 Material and methods

Identification of the *Plutella xylostella* SSRs

I downloaded 171,262 non-redundant unigene sequences of the *P. xylostella* transcriptome from the recently published database (DBM-DB: http://iae.fafu.edu.cn/DBM/) (Tang et al. 2014). Using MIcroSAtellite (MISA) (Thiel et al. 2003), a complete repertoire of SSRs in this dataset was identified with the default settings of motif lengths and minimum repeat numbers, and the incomplete SSRs with a maximum distance of 100 bp between two adjacent complete SSRs. The repeat-based lengths, and the numbers and frequencies of the complete SSRs are summarized in Table 2.1. SSR primers based on the *P. xylostella* transcriptome were then developed using the Primer 3 program (Rozen and Skaletsky 2012) based on flanking sequences.

To identify polymorphic SSRs, I used individuals from three *P. xylostella* strains collected from Fuzhou in China (Fuzhou-S, 26.08°N, 119.28°E) (You et al. 2013), Nagasaki in Japan (Japan-S, 32.80°N, 129.92°E), and Wageningen in the Netherlands (Netherlands-S, 52.00°N, 5.40°E). These colonies were maintained on radish seedlings in a greenhouse at 25 ± 1°C with 16 h LD without exposure to insecticides. These *P. xylostella* samples were individually used for DNA extraction with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The relative purity and concentration of the extracted DNA were estimated with NanoDrop ND-2000 (NanoDrop products, Wilmington, Delaware). The DNA was diluted to a final concentration of 20 ng/μl with double-distilled water.

Based on a total of 281 primer pairs randomly selected from the *P. xylostella* transcriptome dataset, I performed PCR reactions to validate the effective primer pairs using the extracted DNA of the *P. xylostella* larvae from Fuzhou (Fuzhou-S). The forward primers of the validated primer pairs were linked with a universal primer *M-13* (TGT AAA ACG ACG GCC AGT) at their 5' ends.

I used eight individuals from the three *P. xylostella* strains (three individuals from Fuzhou-S, two from Japan-S, and three from Netherlands-S) to identify polymorphic SSRs. A program developed by Schuelke (2000) for PCR was used with the conditions that the primers contained 10 μM reverse primer, 2 μM forward primer with a tail *M-13*, and 8 μM fluorescent-labeled *M-13*. The temperature conditions were at 94°C for 10 min, and then 36 cycles at 94°C for 30 s, 56°C for 45 s, 72°C for 45 s, followed by 8 cycles at 94°C for 30 s, 53°C for 45 s, 72°C for 45 s, and a final extension at 72°C for 10 min. After testing by agarose gel electrophoresis (AGE), sizes of the amplification were detected with ABI 3730 (Applied Biosystems). GeneMapper 4.1 (Applied Biosystems) was used to assign alleles based on the sizes of PCR amplifications. PCR products with an identical size generated by the same pair of primers were considered as an allele. SSR markers that could steadily produce ≥ 2 alleles among the eight individuals were taken to be polymorphic markers.

A total of 288 individuals were collected from nine locations on both sides (Fujian and Taiwan) of the Taiwan Strait, China (Figure 2.1, Table 2.2). These samples were morphologically checked to confirm their identity and kept at -80°C prior to DNA extraction. Genetic analysis was carried out by assaying genotypes of the previously identified polymorphic SSRs. MICRO-CHECKER (Van Oosterhout et al. 2004) was used to determine null alleles of each locus and provide the data on corrected allele frequencies. The selective neutrality of the polymorphic SSRs was evaluated by Ewens-Watterson Test using POPGENE 1.31 (Yeh et al. 1997). Deviations from Hardy-Weinberg equilibrium (HWE) at each locus and for each population were calculated and each linkage among polymorphic SSRs was tested with POPGENE 1.31. The observed heterozygosity and expected heterozygosity were calculated for each locus and each population using FSTAT (Goudet 2001). I also calculated allelic richness per population using ADZE-1.0 (Szpiech et al. 2008), which uses a rarefaction approach to account for differences in sample size. Based on uncorrected and corrected allele frequencies, pairwise genetic differentiations were estimated with F_{ST} (Weir and Cockerham, 1984), and the significance of differentiation being tested using 10,000 permutation steps with Genepop (Rousset 2008). A similar differentiation pattern was found based on uncorrected and corrected allele frequencies (Table 2.3).

Table 2.1 Composition, abundance (number) and frequency of SSRs identified from the *P. xylostella* transcriptome.

SSR	Motif	Length-specific number of SSRs				Total number of SSRs	Frequency of SSRs (%)
		<30 bp	30-39 bp	40-49 bp	≥50 bp		
Monomer	A	2016	7	2	3	2028	16.7
	G	1550	9	3	3	1565	12.9
	C	1445	9	1	1	1456	12.0
	T	1990	7	3	3	2003	16.5
	subtotal	7001	32	9	10	7052	58.0
Dimer	AC/CA	418	6	5	10	439	3.6
	AG/GA	93	0	0	0	93	0.8
	AT/TA	205	0	0	0	205	1.7
	CG/GC	169	0	0	0	169	1.4
	CT/TC	103	0	1	3	107	0.9
	GT/TG	430	7	5	10	452	3.7
	subtotal	1418	13	11	23	1465	12.1
Trimer	AAT/ATA/TAA	218	0	0	0	218	1.8
	TTA/TAT/ATT	203	1	0	0	204	1.7
	CCG/CGC/GCC	647	0	0	0	647	5.3
	GGC/GCG/CGG	627	1	0	0	628	5.2
	Others	1534	2	1	2	1539	12.7
	subtotal	3229	4	1	2	3236	26.6

SSR	Motif	Length-specific number of SSRs				Total number of SSRs	Frequency of SSRs (%)
		<30 bp	30-39 bp	40-49 bp	≥50 bp		
Tetramer	TTTA/TTAT/TATT/ATTT	1	0	0	0	73	0.6
	AAAT/AATA/ATAA/TAAA	58	0	0	1	59	0.5
	Others	174	3	2	3	182	1.5
	subtotal	305	3	2	4	314	2.6
Others	subtotal	66	13	3	3	85	0.7
All SSRs	Total	12019	65	26	42	12152	

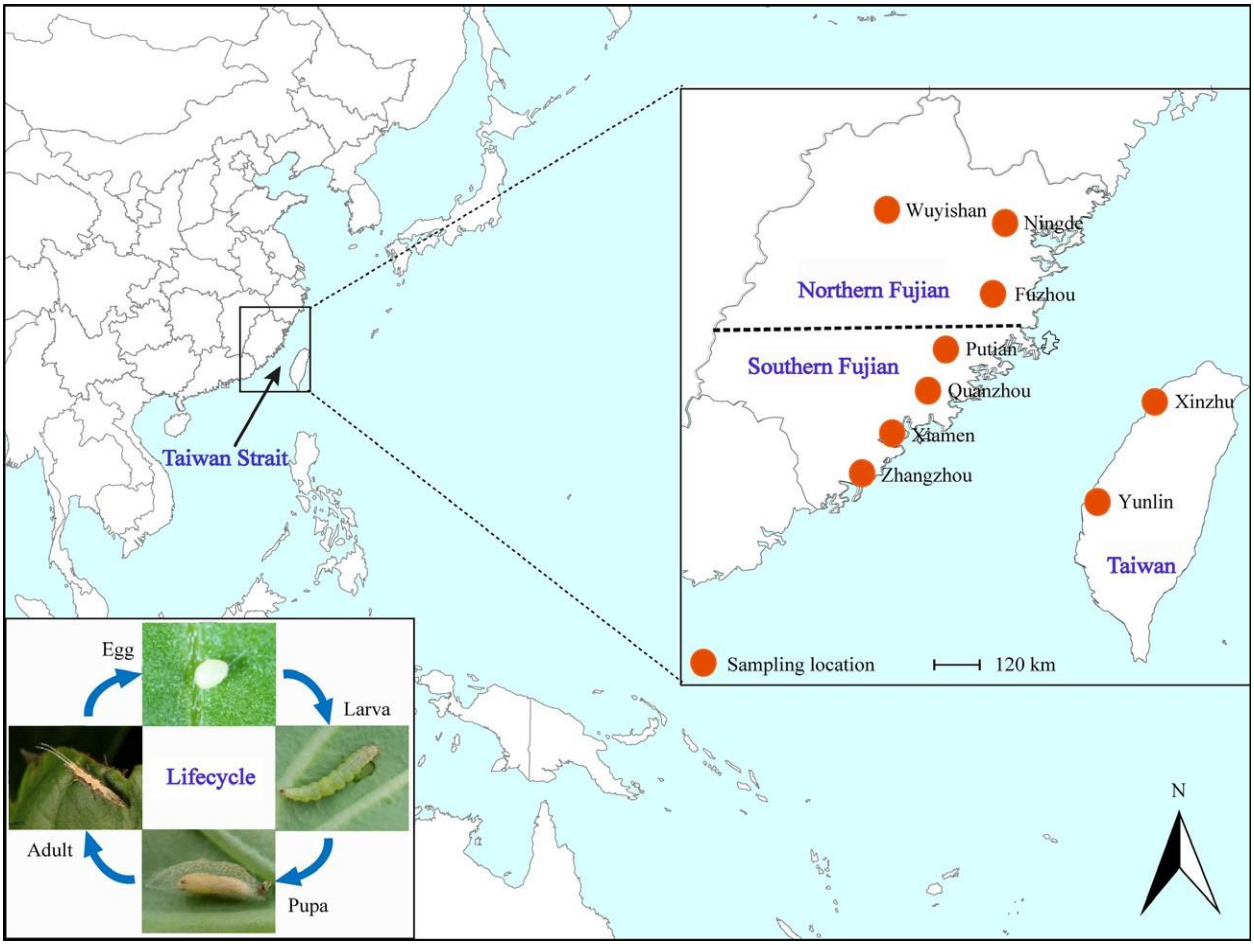


Figure 2.1 Map showing geographic location of the Taiwan Strait (left) and sampling locations of *Plutella xylostella* used for this study. The inset in bottom left corner shows the life cycle of *P. xylostella*. (Photos by Tiansheng Liu).

Table 2.2 Sampling locations, numbers, and collection date of the *Plutella xylostella* (Px) specimens from Fujian and Taiwan, in southeast China.

Region	Sampling location	Geographic coordinates	Px number	Collection date
Northern	Wuyishan	27.70°N, 118.00°E	16	2012.7
Fujian	Ningde	27.13°N, 119.29°E	35	2012.10
	Fuzhou	26.06°N, 119.21°E	42	2011.8
Southern	Putian	25.52°N, 118.80°E	39	2013.10
Fujian	Quanzhou	24.92°N, 118.52°E	37	2013.12
	Xiamen	24.68°N, 118.14°E	32	2014.1
	Zhangzhou	24.04°N, 117.82°E	33	2013.12
Taiwan	Xinzhu	24.91°N, 121.00°E	22	2013.4
	Yunlin	23.72°N, 120.42°E	32	2013.4

Table 2.3 Pairwise differentiation (*F*ST) among the *Plutella xylostella* populations sampled from different locations across the Taiwan Strait based on uncorrected (*a*) and corrected (*b*) allele frequencies.

Sampled locations	Putian	Zhangzhou	Fuzhou	Ningde	Quanzhou	Wuyishan	Xiamen	Xinzhu
(a) Pairwise differentiation (F_{ST}) based on uncorrected allele frequencies								
Zhangzhou	-0.007							
Fuzhou	***0.032***	***0.039***						
Ningde	***0.045***	***0.047***	***0.012***					
Quanzhou	***0.012***	0.011	***0.051***	***0.064***				
Wuyishan	***0.022***	***0.033***	-0.003	***0.007***	***0.045***			
Xiamen	***0.01***	***0.007***	***0.059***	***0.089***	***0.013***	***0.062***		
Xinzhu	-0.012	-0.005	***0.027***	***0.033***	0.011	0.020	***0.020***	
Yunlin	-0.002	0.004	***0.019***	***0.038***	***0.008***	0.016	***0.013***	**-0.003**
(b) Pairwise differentiation (F_{ST}) based on corrected allele frequencies								
Zhangzhou	-0.007							
Fuzhou	***0.029***	***0.038***						
Ningde	***0.041***	***0.044***	***0.006***					
Quanzhou	0.006	0.004	***0.051***	***0.061***				
Wuyishan	***0.022***	***0.034***	-0.003	***0.007***	***0.044***			
Xiamen	***0.01***	0.006	***0.059***	***0.089***	***0.007***	***0.066***		
Xinzhu	-0.012	-0.004	***0.031***	***0.037***	0.005	0.025	***0.019***	
Yunlin	-0.001	0.005	***0.02***	***0.038***	0.001	0.018	***0.013***	-0.002

Numbers in bold italics indicate significant values at $P < 0.001$.

I developed a population-level phylogeny using a neighbor-joining (NJ) method (Saitou and Nei 1987) in POPTREE2 (Takezaki et al. 2010) with 1000 bootstrap iterations. Principal Coordinates Analysis (PCoA) was performed to visualize the genetic differentiation among the *P. xylostella* populations using the standardized covariance method in GenAlEx 6.5 (Peakall and Smouse, 2006) for distance matrix conversion. The population genetic structure and the ancestry proportion of individuals were analyzed using Bayesian clustering method in STRUCTURE (Pritchard et al. 2000) with 50,000 burn-in and a run length of 500,000 Markov chain Monte Carlo (MCMC) repetitions. Sampling location information was used for assisting the clustering (LOCPRIOR model) (Hubisz et al. 2009). For nine locations across the Taiwan Strait, I started with $K = 1$, and ran simulations for K values of 1 through 9 using 20 independent runs. Log-likelihood values of each K and the rate of change in the log probability of data between successive values of K (deltaK) (Evanno et al. 2005) were assessed to determine the optimal genetic clusters using Structure Harvester (Earl and vonHoldt 2012). The optimal genetic clusters were visualized using Distruct (Rosenberg 2004). Hierarchical analyses of molecular variance (AMOVA) among clusters and populations were carried out based on uncorrected allele frequencies using Arlequin 3.01 (Excoffier et al. 2005) to further confirm the population genetic differentiation of the *P. xylostella* populations across the Taiwan Strait. A Mantel test for matrix correlation between genetic distance and geographic distance was performed by using IBDWS (Jensen et al. 2005) with 1000 permutations.

I randomly selected three samples representing different geographic locations, Fuzhou (Northern Fujian), Putian (Southern Fujian), and Yunlin (Taiwan), as cases to estimate the migration rate among populations at the regional level. Population size and migration among populations were analyzed based on Bayesian inference using Migrate 3.6.4 (Beerli and Felsenstein 2001; Beerli 2006), which uses a MCMC approach to approximate the posterior of the parameters. Mutation-scaled population size (θ) was estimated by the equation $\theta = 4Ne\mu$ (where Ne is the long-term [inbreeding] effective population size, μ is the mutation rate per site and generation), and the mutation-scaled migration size (M) was estimated by $M = m/\mu$ (where m is the migration rate per

generation). I gradually increased the numbers in Markov chain settings until smooth histograms were observed and modes were within the 50% credibility intervals. The MCMC-run consisted of a long chain with 5000 recorded steps, 10 concurrent chains (replicates), and 1000 discarded trees per chain. Static heating scheme was also used with four chains of temperature (10,000, 3, 1.5, and 1) with swap- ping interval of 1.

2.3 Results

Characterization of the *Plutella xylostella* SSRs

A total of 12,152 SSRs were identified from the *P. xylostella* transcriptome (~94 Mb), with an average of 129 SSRs per Mb. Approximately 95% of the complete SSRs were shorter than 30 bp in length, while less than 0.1% were longer than 50 bp. In terms of the SSR composition, the numbers of motif- and length-specific SSRs were unevenly distributed. Monomers were the most abundant motifs with a frequency of 58.0%, followed by the trimers (26.6%), dimers (12.1%), and tetramers (2.6%) (Table 2.1).

Based on the PCR validation of 281 primer pairs, 30 pairs of primers produced expected amplicons. High-quality bands in all of the three *P. xylostella* strains (Fuzhou-S, Japan-S, and Netherlands-S) were generated for 15 SSR loci, among which six were monomorphic and nine polymorphic with trinucleotide repeats (Table 2.4).

Table 2*4* **Characteristics of nine polymorphic SSRs developed in *Plutella xylostella*.**

Polymorphic SSR	GenBank Accession No.	Motif	Primers (5'-3')	*Na*	Observed size (bp)	Unigene /Position in the transcripts/Annotation
A-DBM-16	KM925133	ATC	F: GTTCGACATCGGCAGAATTT R: TGGAATTTATGTATCAGCCCAA	15	184–238	Unigene34680_All/UTR
A-DBM-133	KJ701764	CCG	F: TTTAGTGACGAGATGAGCGG R: AGGAATGATGGCAGAAATGG	12	135–177	Unigene99000_All/CDS/ Px013469 (unknown function)
A-DBM-142	KJ701765	TGG	F: GTGCGTCAAATGTCTTGGTG R: CCTATTTGTTGCGGTCCTGT	9	150–174	Unigene26450_All/UTR
B-DBM-1	KJ701767	AAC	F: CAACAAACACAACGGCAATC R: CTGGTATGTCTCCTGACGCA	8	221–290	Unigene48948_All/CDS/ Transcriptional activator cubitus interruptus
B-DBM-23	KJ701768	CCA	F: TGGCTCCACTCCACAACATA R: CCGTGTCGATGGTTTTGTCT	6	219–234	Unigene145643_All/ CDS/ Microtubule-associated protein futsch
B-DBM-25	KJ701769	CCA	F: TACAACACCCAACATGCACC R: TGCTTGTCTTGGATACTGCG	8	104–167	Unigene56663_All/ CDS/ Microtubule-associated protein futsch
B-DBM-30	KM925134	CGC	F: TGCTTATAGCCTCGTAGCCG R: TGAACATCTAGCGGGAGGAC	13	138–177	Unigene113679_All/UTR
B-DBM-34	KJ701770	CTA	F: CCTCATTTGTCCCATCATCC R: CCGAATGGACGAAAACTGAT	10	131–182	Unigene169897_All/UTR
B-DBM-64	KJ701771	AAT	F: TCGCCACGATATGTTCGATA R: AGTTGCATTTACAAGCTCCG	7	153–171	Unigene82431_All/UTR

The annotation information is from DBM-DB (Tang et al. 2014); UTR means untranslated regions; CDS denotes coding sequence. F and R indicate forward and reverse.

Genetic patterns of the *Plutella xylostella* populations across the Taiwan Strait

Using the nine polymorphic SSR loci, a total of 88 alleles were found in the 288 individuals, with the number of alleles per locus ranging from 6 (*B-DBM-23*) to 15 (*A-DBM-16*) and an average of 9.78 (Table 2.4). The observed fixation indexes of all of the identified polymorphic SSRs fell within the 95% confidence interval of theoretical expectation (Table 2.5), suggesting that the hypothesis for neutral selection could not be rejected for any of these loci. Among the 81 HWE tests performed on the nine SSR loci and nine populations, 27 showed significant deviations from equilibrium (Fisher's method, $P < 0.05$), but they were not necessarily associated with particular populations and/or loci. Null alleles were detected in 22 of the 81 loci as a result of heterozygote deficiency (showing a significant positive F_{IS} value, Table 2.6), 20 of which were associated with HWE deviation. It is likely that the presence of null alleles of each locus was responsible for significant HWE deviations and significantly positive F_{IS} values (Brookfield 1996; Endersby et al. 2006).

Our analysis showed that *B-DBM-23* and *B-DBM-25* exhibited linkage disequilibrium in all nine *P. xylostella* populations. These two loci were located at scaffold 89 in the published DBM genome (You et al. 2013) and encoded the same protein, which implied the underlying mechanism associated with their linkage disequilibrium. I therefore removed *B-DBM-25* from the rest of the analyses, meaning that the following analyses were completed on eight SSR loci. Across the different sampled locations, the number of alleles ranged from 28 in Wuyishan to 52 in Fuzhou. The average expected heterozygosity (*H*e) ranged from 0.47 in Wuyishan to 0.58 in Zhangzhou, and the allelic richness ranged from 3.50 in Wuyishan to 5.25 in Xiamen. A total of 23 population-specific alleles were identified (Table 2.6).

Table 2.5 Analysis for the selective neutrality of the identified polymorphic SSR loci based on Ewens–Watterson Test using POPGENE.

Locus	*N*[2]	OF[3]	Mean[1]	SE[1,4]	*L95*[1,5]	*U95*[1,6]
B-DBM-34	576	0.32	0.37	0.02	0.19	0.75
B-DBM-25	576	0.49	0.44	0.03	0.22	0.82
B-DBM-23	576	0.51	0.52	0.03	0.26	0.92
B-DBM-30	576	0.3	0.3	0.01	0.15	0.61
A-DBM-16	576	0.44	0.26	0.01	0.14	0.51
B-DBM-1	576	0.59	0.43	0.02	0.22	0.79
B-DBM-64	576	0.64	0.48	0.03	0.23	0.87
A-DBM-142	576	0.59	0.4	0.02	0.2	0.75
A-DBM-133	576	0.23	0.32	0.02	0.17	0.67

[1]These statistics were calculated using 1000 simulated samples.
[2]The total number of alleles.
[3]Observed sum of the square of allelic frequency.
[4]Standard error of the mean.
[5]Lower 95% confidence limit.
[6]Upper 95% confidence limit.

Table 2.6 Genetic diversity at eight microsatellite loci for the sampled *Plutella xylostella* populations across the Taiwan Strait.

	Wuyishan		Ningde		Fuzhou		Putian		Quanzhou		Xiamen		Zhangzhou		Xinzhu		Yunlin	
Loci	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*
B-DBM-34	0.88	0.67	0.63	0.73	0.29	0.66	0.38	***0.66***	0.27	***0.64***	0.38	***0.54***	0.45	***0.71***	0.59	0.69	0.63	0.74
B-DBM-23	0.56	0.54	0.49	0.6	0.36	0.49	0.44	0.52	0.27	***0.39***	0.44	0.39	0.45	0.5	0.5	0.52	0.41	0.46
B-DBM-30	0.69	0.67	0.49	0.58	0.69	0.68	0.51	***0.7***	0.7	0.74	0.69	0.77	0.55	***0.71***	0.64	0.65	0.69	0.72
A-DBM-16	0.06	0.06	0.06	0.06	0.05	0.05	0.41	***0.69***	0.57	***0.75***	0.44	***0.8***	0.42	***0.72***	0.36	***0.67***	0.56	0.6
B-DBM-1	0.31	0.42	0.31	0.36	0.48	0.53	0.26	***0.43***	0.3	0.34	0.28	***0.4***	0.39	0.44	0.32	0.36	0.31	0.38
B-DBM-64	0.31	0.29	0.34	0.34	0.29	0.37	0.26	0.28	0.22	***0.43***	0.31	***0.42***	0.27	0.36	0.32	0.35	0.25	***0.38***
A-DBM-142	0.31	0.35	0.4	0.38	0.43	0.44	0.38	0.37	0.49	0.48	0.34	0.43	0.36	0.44	0.27	0.24	0.41	0.41
A-DBM-133	0.88	0.77	0.83	0.75	0.88	0.78	0.64	***0.78***	0.7	0.81	0.53	***0.74***	0.67	0.72	0.68	0.75	0.75	0.77
Mean	0.5	0.47	0.44	0.48	0.43	***0.5***	0.41	***0.55***	0.44	***0.57***	0.43	***0.56***	0.45	***0.58***	0.46	***0.53***	0.5	***0.56***
Total alleles	28		40		52		44		46		46		46		40		45	
Allelic richness	3.5		4.33		5.05		4.91		5.2		5.25		5.07		4.86		5.12	
Specific	2		4		4		3		2		3		2		1		2	

Ho denotes observed heterozygosity; He refers to expected heterozygosity; He in bold italic indicates a significant positive Fis value (heterozygote deficiency) with $P < 0.05$ based on 1440 randomizations.

The *P. xylostella* populations across the Taiwan Strait exhibited genetic differentiation among different sampled locations. Based on the Bayesian cluster analysis, the optimal number of clusters was identified to be K = 2, and each of the 288 individuals was thus proportionally assigned to the two clusters (Figure 2.2) composed of (1) South Fujian and Taiwan (including Putian, Quanzhou, Xiamen, Zhangzhou, Xinzhu, and Yunlin), and (2) Northern Fujian (including Wuyishan, Ningde, and Fuzhou). Three-level hierarchical AMOVA analysis supported the result of the Bayesian cluster analysis with two genetic clusters (df = 1, percentage of variation = 3.65%, $P = 0.0068$). Similar patterns were observed using population-level phylogenetic analysis (Figure 2.3A). These results were further verified through the PCoA analysis (Figure 2.3B). Analysis of the *P. xylostella* populations of Fujian showed that the genetic distance significantly increased with the geographic distance (Figure 2.4), which indicated that more genetically similar relationships were found for nearby populations than those of more distant populations.

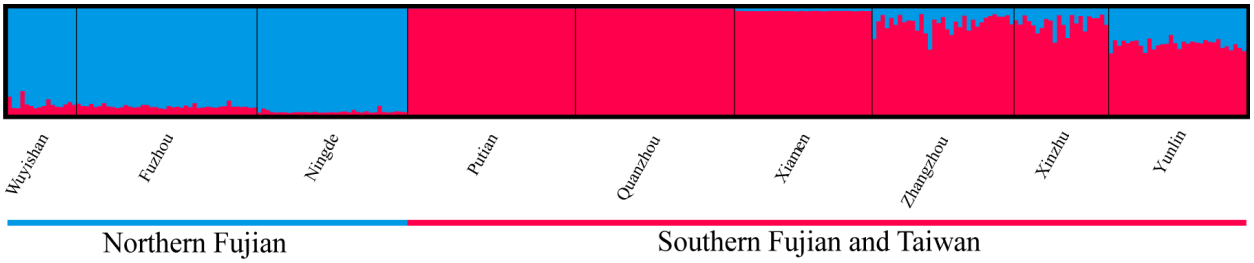


Figure 2.2 Population structure plot showing two distinct clusters of the *Plutella xylostella* populations sampled from nine different locations across the Taiwan Strait. Individuals are indicated by vertical bars with different colors to denote the membership of location-associated populations.

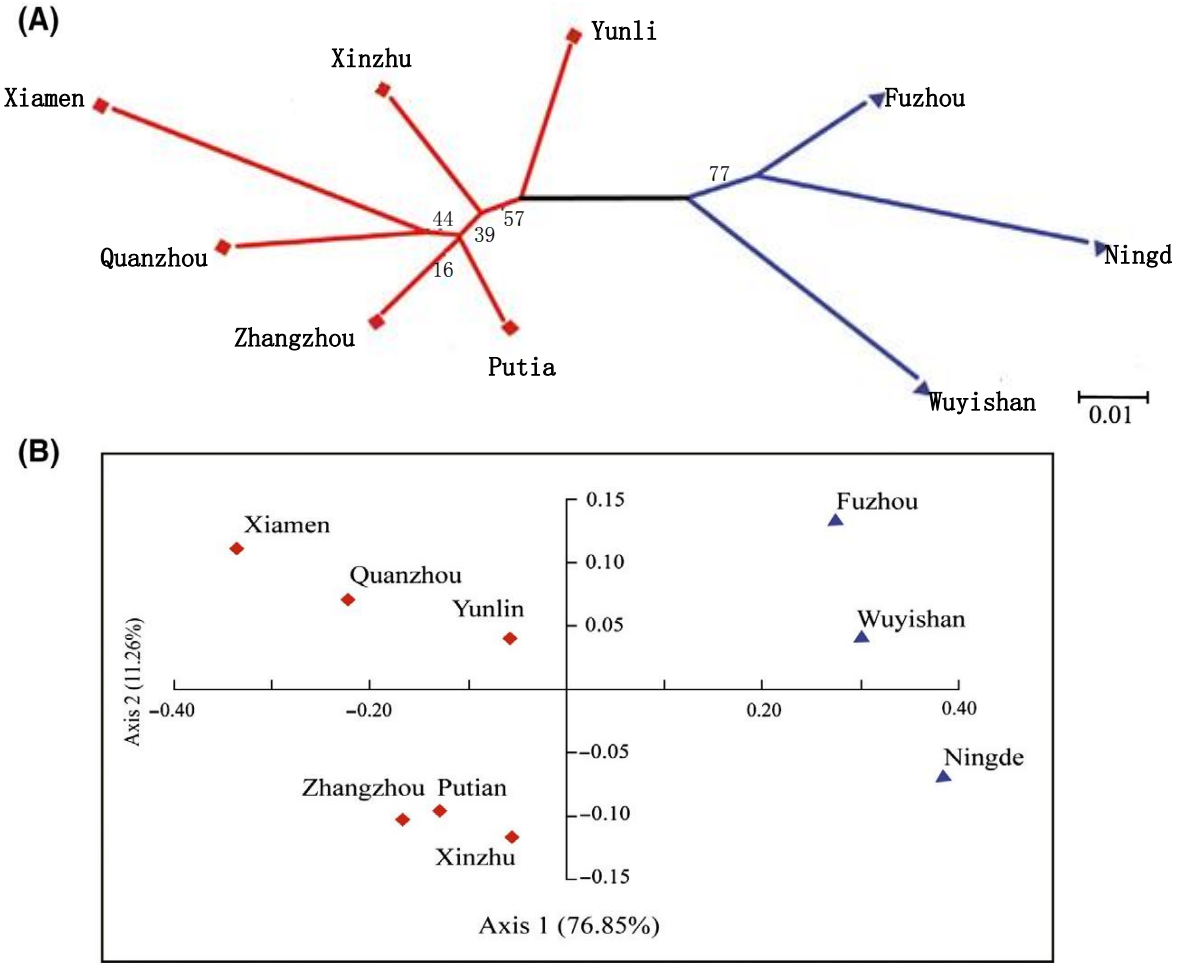


Figure 2.3 A neighbor-joining tree based on 1000 bootstraps (A) and Principal Coordinates Analysis (B) of the *Plutella xylostella* populations sampled from different locations in Fujian and Taiwan. Two groups (K = 2) are intuitively clustered with colored triangles and diamonds to indicate the membership of location-associated populations.

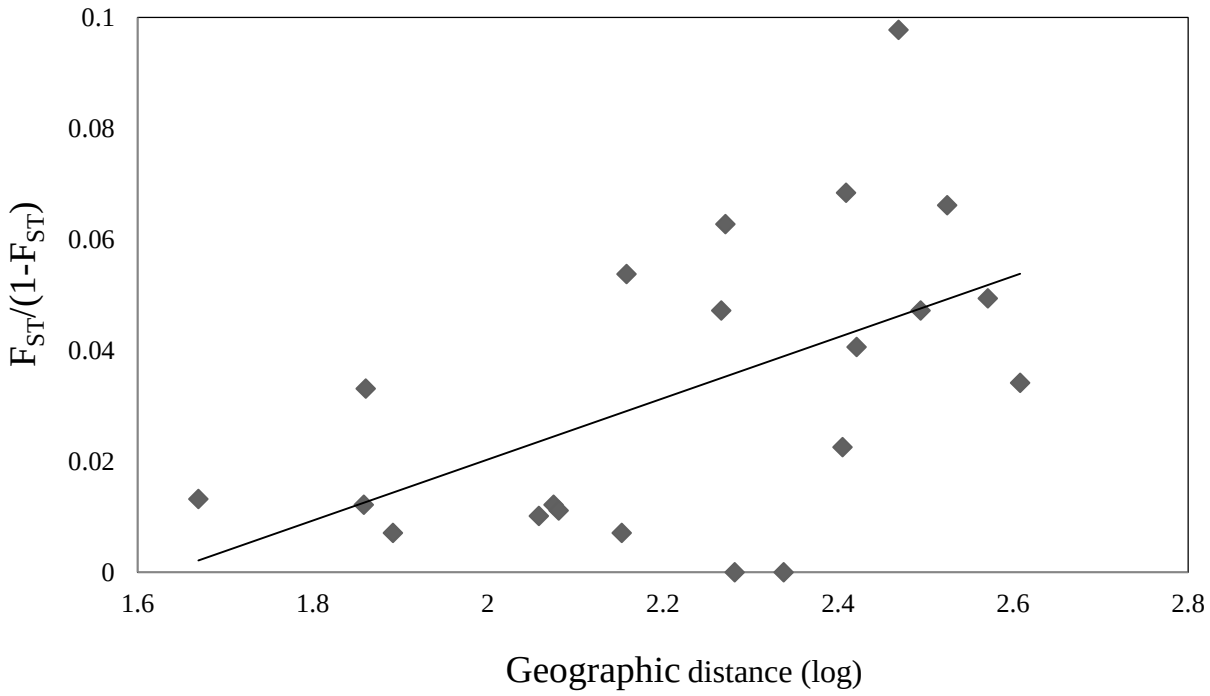


Figure 2.4 Regression analysis between the geographic distance (log) and genetic distance ($F_{ST}/(1-F_{ST})$) among the *Plutella xylostella* populations sampled from different locations in Fujian province (R^2=0.289; P=0.028).

Table 2.7 Mutation-scaled population sizes (θ) and migration rates (M) among the *Plutella xylostella* populations sampled from Fuzhou, Putian, and Yunlin, estimated with Migrate.

	Location		Percentiles		
Parameter	From	To	2.50%	97.50%	Median
θ		Putian	0.094	0.1	0.098
θ		Fuzhou	0.095	0.1	0.098
θ		Yunlin	0.094	0.1	0.098
M	Fuzhou	Putian	34	82.667	59.667
M	Yunlin	Putian	30	76.667	54.333
M	Putian	Fuzhou	27.333	70.667	50.333
M	Yunlin	Fuzhou	18.667	62.667	41.667
M	Putian	Yunlin	49.333	100.667	75.667
M	Fuzhou	Yunlin	24	80.667	52.333

The pairwise F_{ST} values were low to moderate with a maximum between Xiamen and Ningde (0.089), which indicated a high level of movement among populations. The differentiation values between clusters (cluster I vs. cluster II) were generally higher than those within clusters (Table 2.3). The mutation-scaled population sizes (θ) of the sample populations were similar, and mutation-scaled migration rates (M) estimated with Migrate showed high gene flow among different geographic regions, with the highest value between Putian (Southern Fujian) and Yunlin (Taiwan) (Table 2.7).

2.4 Discussion

Identification of SSR markers from the *Plutella xylostella* transcriptome

Conventional methods of microsatellite identification from partial genomic libraries have proven to be inefficient for some taxa such as the Lepidoptera (Zhang 2004). Low abundance of SSRs, existence of microsatellite DNA families (microsatellite sequences with similar or almost identical flanking regions) and polymorphism of the flanking regions, which cause the failure of amplification in lepidopteran genomes may be associated with low isolation efficiency of SSR markers via traditional laboratory approaches (Ji et al. 2003; Meglecz et al. 2004; Zhang 2004; Meglecz et al. 2007). It is possible to justify the low amplification efficiency by assuming polymorphic flanking regions of microsatellite loci in *P. xylostella*, suggested by the heterozygous nature of the recently published genome of this species (You et al. 2013). Such a hypothetical explanation is supported by observed single nucleotide polymorphisms (SNPs) for several flanking regions of the same microsatellite locus in *P. xylostella* (data not shown). Based on the 281 selected primer pairs, I found that 30 primer pairs could amplify expected sizes in the Fuzhou strain, of which 15 SSR loci showed effective bands in all three *P. xylostella* strains collected from different countries, while others failed and may be related to the polymorphic flanking regions presented in different *P. xylostella* strains.

Microsatellites can be under selection as these repeats may have functions such as regulation of gene activities (Li et al. 2002). The neutrality of microsatellites should therefore be tested before being used in answering ecological questions such as the

significance of dispersal (Selkoe and Toonen 2006). No selection was detected in the remaining eight loci, which indicated that these markers were desirable for the analysis of neutral genetic variation in the *P. xylostella* populations.

Genetic variation of the *Plutella xylostella* populations

Using the eight successfully genotyped polymorphic SSR loci, the initial analysis of the nine populations showed that overall genetic diversity of these *P. xylostella* populations was higher than that of other insect species, such as *Nilaparvata lugens* (Jing et al. 2012) and *Diabrotica virgifera* (Kim et al. 2008) using similar molecular markers. Romiguier et al. (2014) investigated the genetic diversity of 76 nonmodel animal species by sequencing their transcriptomes, and show that short-lived or highly fecund species are genetically more diverse than the long-lived or low-fecundity species with brooding ability. *P. xylostella* is an insect pest with high fecundity and short developmental duration (up to 19 generations per year in Fujian and Taiwan, You and Wei 2007), which may contribute to this higher population genetic diversity compared with other insect species (Kim et al. 2008; Jing et al. 2012). However, compared with other studies analyzing *P. xylostella* population using genomic SSR loci (Endersby et al. 2006; Wei et al. 2013), our results show low diversity, possibly due to the conservativeness of the SSR markers isolated from the transcriptome (Kim et al. 2008; Wang et al. 2014).

The effectiveness of these polymorphic microsatellite markers in identifying weak but significant genetic structure of *P. xylostella* was important in defining two main clusters among populations across the Taiwan Strait. The first cluster included populations collected from Southern Fujian and Taiwan and the second cluster consisted of populations sampled from Northern Fujian. Despite the fact that the populations were collected at different dates, I believe that these clusters are accurate and independent of collection dates. In Australia and New Zealand, high genetic similarity across the *P. xylostella* populations was found over a couple of years (2001-2003) and could be attributed to gene flows originating from frequent vegetable transportation (Voice and Chapman 2000) and prevailing winds (Endersby et al. 2006). In China, Fu et al. (2014) show, using light-trapping observations, that movements of *P. xylostella* across the Bohai Gulf are

consistent over a period of 11 years, most likely contributing to a stable and consistent pattern of gene flow, which was coincident with genetic similarity between populations from Central China and populations from Northeast China as reported by two independent investigations (Wei et al. 2013; Yang et al. 2015). These pieces of evidence suggest that these clusters are unlikely to be an artefact of different sampling dates.

The genetic similarity among populations of Southern Fujian and Taiwan in our first cluster suggests that air-flow across the Taiwan Strait might be the main factor for genetic similarity among populations, with dominant winds being southwestward from June to August and northeastward from September to April (Hwang et al. 2006), linking Southern Fujian to Taiwan and vice versa. Such a meteorological pattern favors the formation of genetically similar *P. xylostella* populations in cluster one by homogenizing genetic variation through gene flow. These winds do not connect populations in Northern Fujian with populations in Southern Fujian and Taiwan, which may explain the differentiation of the two clusters.

When our analysis was restricted to the *P. xylostella* populations of the Fujian province, nearby local populations were genetically more similar than populations isolated by longer geographic distances. In addition, while dominant winds across the Taiwan Strait did not contribute to gene flow between some of the populations, they showed high genetic similarity (i.e., populations within Southern Fujian) (Figure 2.1). I believe that this may be linked to transportation of vegetables and other plant products (Delgado and Cook 2009; Boykin et al. 2010; Niu et al. 2014). In Fujian, a majority of agricultural products are supplied by small-scale farms and usually at the local or regional scale (Rao 2012). Large numbers of rural areas produce their own vegetables and are self-sufficient. Urban areas such as Xiamen and Fuzhou, however, must import vegetables from various nearby counties, which raise the possibility of this pest being transported to urban centers, where it also may be mixed. Such conditions allow for gene flow among nearby populations.

At the same time, our results showed that, in the first cluster, genetic diversity within

each population was generally higher than in populations of the second cluster. On the contrary, lower numbers of specific alleles were generally found in populations of cluster one when compared with those populations in cluster two (except population Wuyishan due to small sample size). Gene flow mediated by large-scale movements can also shape genetic variation within populations (Freeland 2006; Kremer et al. 2012; Raymond et al. 2013; Pierce et al. 2014). Another aspect that should be considered when examining genetic diversity within these two clusters is that both Fujian and Taiwan regions possess year-round intensive *Brassica* crop production. The presence of persistent populations of *P. xylostella* in these regions (You and Wei 2007) can contribute to the continuous accumulation of mutations. New mutations accumulated in local populations and higher levels of dispersal thus may significantly increase genetic diversity in Southern Fujian and Taiwan populations compared with Northern Fujian populations, where gene flow with other regions is relatively low.

2.5 Conclusion

The diamondback moth is an insect pest with a worldwide distribution, with short- to long-distance dispersal capability. Our analysis shows that several factors can play a role in defining genetic variation and structure at both local and regional levels. Our results support the fundamental role of air currents in intermixing *P. xylostella* populations from southern Fujian and Taiwan, and that vegetable transportation among rural and urban centers may enhance the complexity of gene flow. In terms of factors affecting population genetic structure at local to regional scales, this complexity may not always be recognized as an important force shaping population genetic diversity of insect pests. Further studies, using landscape genetics and information-theoretical selection models may help to disentangle the influence of these various mechanisms in governing the gene flow in DBM from local to regional levels.

Chapter 3 Herbivore invasion triggers adaptation in a newly associated third trophic level species and shared microbial symbionts, a case study based on phylogeographic analysis of *Plutella xylostella* and *Cotesia vestalis*

3.1 Introduction

Many crop pathogens and pests have recently expanded their ranges due to human activities and climate change, and this is likely to continue despite increasing quarantine efforts (Bebber 2015). Impacts of biological invasion manifest at scales ranging from genetic and evolutionary changes in individuals to ecosystems and landscapes (Pejchar and Mooney 2009; Ehrenfeld 2010). In the course of invasion, the population dynamics and evolutionary processes of local flora and fauna may be affected through interaction between them and invaders (Strauss et al. 2006; Bezemer et al. 2014; Pintor and Byers 2015). Many factors, including genetic architecture and variation of local populations determine the capacity of native species to form new interactions (Strauss et al. 2006).

Phylogeographic analysis of intraspecific genetic variation can be used to explore the evolutionary history of a species, provide evidence of its geographical origin, and of patterns of expansion (Oliveira et al. 2013; Sproul et al. 2014; Rewicz et al. 2015). Comparative phylogeography aims to examine the temporal (evolutionary) and spatial (biogeographic) effects on genetic structure of closely related species (Papadopoulou et al. 2009; Nicholls et al. 2010, Avise et al. 2016). This type of study can help reveal the impacts of biological invasions on local communities over wide spatial scales. Higher trophic levels, such as parasitoids, in a given location may switch among taxonomically disparate, but ecologically similar, sets of hosts by ecological sorting (Weiher and Keddy 2001), resulting in different origins and timings of range expansion being represented in the new assemblages (Althoff 2008).

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is a *Brassica*-specialist herbivore of global significance (Talekar and Shelton 1993; Furlong, et al. 2013; You, et al. 2013; Li, et al. 2015). It invaded many regions (i.e. East Asia, Oceania and North America) in recent centuries, most likely due to human activities, such as globalization of *Brassica* crops (Hori K

1910; Kfir 1998; Capinera 2000). In recent studies of *P. xylostella*, genetic homogeneity has been found in many populations across Asia-Pacific regions (Endersby et al. 2006; Wei et al. 2013; Yang et al. 2015). Air flow and transportation of agricultural products have been proposed as the main reasons for high levels of gene flow (Endersby et al. 2006; Delgado and Cook 2009; Wei et al. 2013; Fu et al. 2014; Niu et al. 2014; Ke et al. 2015).

A broad range of natural enemies, including parasitoids, arthropod predators, pathogenic fungi, and bacteria have been recorded to attack *P. xylostella* (Talekar and Shelton 1993; Furlong, et al. 2013; Li, et al. 2015). *Cotesia vestalis* (=*plutellae*) Haliday (Hymenoptera: Braconidae) is one of the most important biocontrol agents of *P. xylostella* (Delvare et al. 2004; Furlong et al. 2013; Li et al. 2015) and occurs in 38 countries (Furlong et al. 2013). Whilst *C. vestalis* has been introduced to Australia, North America, and the Caribbean in over 20 classical biological control programs (Talekar and Shelton 1993; Shelton 2004), there are no records of it being introduced to Japan, Vietnam, Malaysia (Cameron Highlands) or China (Ooi 1992; Alvi and Momoi 1994; Liu, et al. 2000). Yet, *C. vestalis* is reported to be among the most predominant parasitoids of *P. xylostella* across East Asia (Liu et al. 2000; Shi and Liu 2003; Shi et al. 2004) due to its tolerance to high temperatures (Verkerk and Wright 1997). Like most parasitoids, *C. vestalis* is not adapted to long-distance migration (Talekar and Shelton 1993), but its widespread use in classical biological control and unintended dispersal by trade in vegetables suggests that high genetic similarity among *C. vestalis* populations would not be unexpected.

In this study, I analyzed the phylogeographic systems of *P. xylostella* and *C. vestalis* in East Asia based on a set of mitochondrial genes. Two key questions were addressed: (i) How did *P. xylostella* and *C. vestalis* populations spread during their evolutionary history? (ii) What is the most parsimonious explanation for currently observed interactions between *P. xylostella* and *C. vestalis*? To address these questions, I considered the effects of inter-specific horizontal transfer of *Wolbachia*, a genus of bacteria that is inherited in the cytoplasm and can cause feminization, parthenogenesis and induction of reproductive incompatibility in the host (Werren 1997). I analyzed infection of *Wolbachia* and genetic diversity for our sampling populations, and demonstrated phylogeographic relationships giving insights into the demographic histories of both species.

3.2 Materials and methods

Sample collection and species identification

I collected *P. xylostella* and *C. vestalis* from the same or nearby cabbage and broccoli fields in East Asia between 2012-2014 (Table 3.1). Twenty-nine samples of *P. xylostella* and *C. vestalis* were collected from the same sites, one sample of *P. xylostella* was collected in Chongqing (CQ) and its counterpart *C. vestalis* sample was obtained in Luzhou Sichuan (SCLZ) near CQ. One additional *C. vestalis* sample from Mozambique in Africa was included for a global phylogenetic tree construction (see below). *P. xylostella* pupae and adults, and *C. vestalis* cocoons were morphologically identified and preserved in 95% ethanol. Second and third instar *P. xylostella* larvae were maintained on cruciferous vegetable leaves (collected from the field where the insect individuals were sampled) for parasitoid emergence, and individuals were then preserved in 95% ethanol. Specimens were stored at -80℃ prior to DNA extraction. A total of 323 *P. xylostella* and 326 *C. vestalis* individuals were used in this study. I used a 600 bp mitochondrial gene sequence (COI) (Table 3.2) and DNA barcoding criteria to identify insect species using BOLD (Ratnasingham and Hebert 2007) to confirm the species identity of *C. vestalis*. The same procedures were performed for *P. xylostella* individuals.

Table 3.1. Details of *Plutella xylostella* and *Cotesia vestalis* samples

Sample location	Number of samples of P.x. |C.v	Latitude	Longitude	Host plants	Sampling date
Changchun, Jilin, China (JLCC)	18|14	43.862	125.326	Cabbage	2013.7
Shenyang, Liaoning, China (LNSY)	17|21	41.554	123.299	Cauliflower and Turnip	2013.9
Beijing, China (BJ)	8|4	40.031	116.279	Turnip	2012.1
Tianjing, China (TJ)	4|22	39.363	117.734	Chinese cabbage and Turnip	2013.9
Qingdao, Shandong, China (SDQD)	19|14	36.306	120.399	Cauliflower and Cabbage	2013.6, 2014.9
Shangluo, Shaanxi, China (SXSL)	8|2	33.870	109.939	Cabbage	2013.5
Zhengzhou, Henan, China (HNZZ)	20|20	34.868	113.624	Cabbage	2013.7
Shanghai, China (SH)	15|19	30.902	121.397	Cauliflower and Cabbage	2012.10, 2014.5, 2014.9
Hefei, Anhui, China (AHHF)	8|4	31.822	117.228	Chinese cabbage	2012.11
Wuhan, Hubei, China (HBWH)	16|10	30.486	114.472	Cabbage	2014.5
Luzhou, Sichuan, China (SCLZ)	na|8	28.874	105.447	Cauliflower	2012.11
Chongqing, China (CQ)	7|na	30.810	108.399	Cabbage	2012.1
Katmandu, Nepal（NPKT）	7|17	27.685	85.365	Cabbage	2013.9
Nanchang, Jiangxi, China (JXNC)	14|14	28.7231	115.916	Chinese cabbage	2014.6
Guiyang, Guizhou, China (GZGY)	17|12	26.458	106.600	Cabbage	2012.1
Fuzhou, Fujian, China (FJFZ)	13|19	26.010	119.238	Cauliflower and Chinese cabbage	2014.3
Putian, Fujian, China (FJPT)	8|3	24.922	118.517	Cauliflower and Cabbage	2013.12
Quanzhou, Fujian, China (FJQZ)	11|10	24.036	117.815	Cabbage	2013.12
Xiamen, Fujian, China (FJXM)	7|14	25.359	119.041	Cauliflower and Cabbage	2013.11

Sample location	Number of samples of P.x. |C.v	Latitude	Longitude	Host plants	Sampling date
Zhangzhou, Fujian, China (FJZZ)	7|3	24.681	118.139	Cauliflower and Cabbage	2013.12
Yuxi, Yunnan, China (YNYX)	15|14	24.109	102.758	Cauliflower and Cabbage	2012.11, 2014.6
Guangzhou, Guangdong, China (GDGZ)	17|17	23.123	113.332	Cauliflower and Cabbage	2012.11, 2014.6
Nanning, Guangxi, China (GXNN)	14|19	22.862	108.301	Chinese cabbage	2012.1
Phetchabun, Thailand (TLPH)	7|10	16.417	101.190	Cabbage	2013.7
Dalat, Vietnam (VTDL)	14|14	11.958	108.420	Cauliflower and Cabbage	2013.8
Shihezi, Xinjiang, China, (XJSHZ)	4|1	44.308	86.006	Cabbage	2013.8
Jiuquan, Gansu, China (GSJQ)	5|1	40.133	94.649	Cauliflower	2012.8
Zhongwei, Ningxia, China (NXZW)	1|1	37.475	105.690	Oilseed rape	2013.8
Yinchuan, Ningxia, China (NXYC)	na|1	38.628	106.066	Cabbage	2013.8
Cameron highland, Malaysia (MLCH）	11|13	3.9380	102.420	Cauliflower	2013.11
Kota Kinabalu, Malaysia (MLKK)	12|10	5.9843	116.576	Cabbage	2013.1
Vandyzi, Manica, Mozambique (MZVM)	na|2	-18.929	33.180	Cabbage	2014.3

Sample size of *P. xylostella* and *C. vestalis* are indicated on left- and right-hand side of "|", respectively. "na" represents no specimens were used.

Table 3.2 Information of the gene fragments and related primers used in *P. xylostella* and *C. vestalis*

	Gene fragment	Primers	Primer sequences (5'-3')	Annealing temperature (℃)	Fragment length (bp)	Reference
DBM	*COI*	DBM-*COI*-F	AAATTTACAATTTATCGCTTAATCTCAGCC	55	800-1000	Yukuhiro *et al.*, 2002
		DBM-*COI*-R	CCTTTTCTTGTGTAATAATATGGAAATTATACC			Yukuhiro *et al.*, 2002
	Cytb	DBM-*cytb*-2F	ACACGCTAATGGAGCATC	60	550-600	This study
		DBM-*cytb*-2R	CTGGTTGAATGTGAATAGGA			This study
	NadhI	DBM-*nadhI*-2F	ATCATAACGATAACGAGG	55	730-770	This study
		DBM-*nadhI*-2R	CAAATTCGTAAAGGTCCT			This study
CV	*CO1*	CV-*COI*-F	GGTCAACAAATCATA AAGATATTGG	58	650-700	Folmer *et al.*, 1994
		CV-*COI*-R	TAAACTTCAGGGTGACCAAAAATCA			Folmer *et al.*, 1994
	Cytb	CV-*cytb*-F	TATGTACTACCATGAGGACAAATATC	50	460-500	Simon *et al.*, 1994
		CV-*cytb*-R	ATTACACCTCCTAATTTATTAGGAAT			Simon *et al.*, 1994
		CV-*cytb*-2F	CGAACTACCAACACCAATTA	58	900-1000	This study
		CV-*cytb*-2R	TGGGTATTCTACAGGTTGAG			This study
	NadhI	CV-*nadhI*-F	ACTAATTCAGATTCTCCTTCT	50	460-500	Smith and Kambhampati, 1999
		CV-*nadhI*-R	CAACCTTTTAGTGATGC			Smith *et al.*, 1999
		CV-*nadhI*-5F	TTCGAGGCAAAGTTATTC	55	700-750	This study
		CV-*nadhI*-7R	ATTATCGGAAAGGACCTA			This study
Wolbachia	*wsp*	*wsp*81F	TGGTCCAATAAGTGATGAAGAAAC	50.5	550-600	Braig *et al.*, 1998
		*wsp*691R	AAAAATTAAACGCTACTCCA			Braig *et al.*, 1998

DNA Extraction and sequencing

Total genomic DNA was extracted from individual insects using DNeasy Blood and Tissue Kit (Qiagen, Germany). Three mitochondrial genes, *COI*, cytochrome b (*Ctyb*), NADH dehydrogenase subunit I (*NadhI*) were sequenced for both *P. xylostella* and *C. vestalis* (Table 3.2). Primers were developed for *Cytb* and *NadhI* of *P. xylostella* as well as *Cytb* and *NadhI* of *C. vestalis* using Primer Premier Version 5 (Premier Biosoft International, Palo Alto, CA, USA) based on the reference mitochondrial genomes. Primers of other gene segments were based on published references (Table 3.2).

Infection of *P. xylostella* and *C. vestalis* by *Wolbachia* was determined using specific primers to amplify a 600 bp product of the *wsp* gene (Table 3.2). A positive control of PCR reaction (with DNA of *Wolbachia*-infected samples as templates) was conducted when an infection of *Wolbachia* was observed in a sample. Samples with unique PCR products of the expected length were treated as being infected by *Wolbachia*.

PCR was conducted using the Mastercycler pro system (Eppendorf, Germany) under the following conditions: an initial denaturation for 2 min at 94ºC, followed by 35 cycles of 10 s at 96 ºC, 15 s at specific annealing temperature of each genes (Table 3.2), and 1 min at 72 ºC, and a subsequent final extension for 10 min at 72 ºC. Amplified products were purified and bidirectionally sequenced using the ABI 3730xl DNA Analyzer by Sanboyuanzhi Biotechnology Co., Ltd (Beijing, China). All the sequences were deposited in the Genebank database (accession number from KX604356 to KX606864).

Genetic analysis

Sequences of each gene fragment were aligned using MEGA5.2 (Tamura et al. 2011). All mitochondrial sequences for each of the species were aligned independently using MAFFT-7.037 (Katoh and Standley 2013). Conservative regions selected by Gblock-0.91b (Talavera and Castresana 2007) were used for gene concatenation, performed by Sequance-Matrix-1.7.8 with default parameters (Vaidya, et al. 2011). Parameters of genetic diversity, including haplotype diversity and nucleotide diversity were calculated using the DnaSPv5 (Librado and Rozas 2009).

Populations with > 5 individuals were included in the calculation of parameters related to genetic diversity (26 populations of *P. xylostella* and 17 of *C. vestalis*).

Phylogenetic relationships were constructed based on the three combined mitochondrial genes of *P. xylostella* and *C. vestalis*. I also downloaded *COI* sequences of *C. vestalis* and *C. flapvis* from NCBI (http://www.ncbi.nlm.nih. gov/) and constructed a global phylogenetic tree of *C. vestalis.* The phylogeny of *wsp* sequences was also developed. *Wsp* sequences with the best hit (with less than 3 gaps and not less than 99% identity) when blasting the *wsp* sequences from this study were downloaded from NCBI. Accession number and host species were recorded. Additional sequences of the *wsp* gene of alternative hosts (*P. xylostella* or *C. vestalis*) from NCBI were also used.

Phylogenetic inferences were performed using the neighbor-joining (NJ) and maximum likelihood (ML) methods by PAUP*4.0b10 (Swofford 2003). The software MrModeltest version 2.3 (Nylander 2004) was used to assist the selection of the best-fit nucleotide substitution model. The General Time Reversible model was used with Gamma distributed with Invariant sites (GTR+I+G) based on the Akaike Information Criterion (AIC).

Network analysis was conducted for mitochondrial genes of both species using a median-joining algorithm implemented in the software Network, version 4.6.1.3 (Bandelt, et al. 1999). I constructed the haplotype networks of *P. xylostella* and *C. vestalis* for individual genes as well as the combined mitochondrial gene sequences. Haplotype type and frequency for each population were also recorded.

I calculated the *Tajima's D* and *Fu's Fs* for each population (> 5 individuals) of the two species based on the combined mitochondrial genes by using Dnasp V5 (Librado and Rozas 2009). Analyses of mismatch distributions were performed for the two species as well. For *C. vestalis*, these analyses were also performed on three selected clusters based on the phylogenetic tree of three concatenated mitochondrial genes.

BEAST (Drummond and Rambaut 2007) was also used to analyze the coalescent time of lineages in *P. xylostella* and *C. vestalis*, based on *COI* sequences. For *P. xylostella*, more samples from the Old World and Oceania were included for more precise coalescent time inference (Pichon et al. 2006; Saw et al. 2006). As the mutation rates varied among insect lineages, I used the mutation rate reported in Papadopoulou et al. (2009) with a lognormal relaxed clock while estimating the evolutionary timescales.

3.3. Results

Infection by *Wolbachia*

From 323 *P. xylostella* and 326 *C. vestalis* individuals analyzed, 100 *P. xylostella* (infection rate of 30.96%) and 52 *C. vestalis* (infection rate of 15.95%) were identified as infected by *Wolbachia*. The *wsp*-based phylogenetic tree (Figure 3.1) showed that the *Wolbachia* strains were distributed in distinct clades that were not defined by host species. Lineage 4 (*plutWB1*) consists of sequences extracted from *P. xylostella* (YNYX3 PX, MLCH2 PX, MLCH3 PX, NPKT5 PX, and MLCH6 PX) and *C. vestalis* (MCLH1 CV and NPKT14 CV). Different host species including herbivores, parasitoids and a predator were presented in lineage 3 (Figure 3.1).

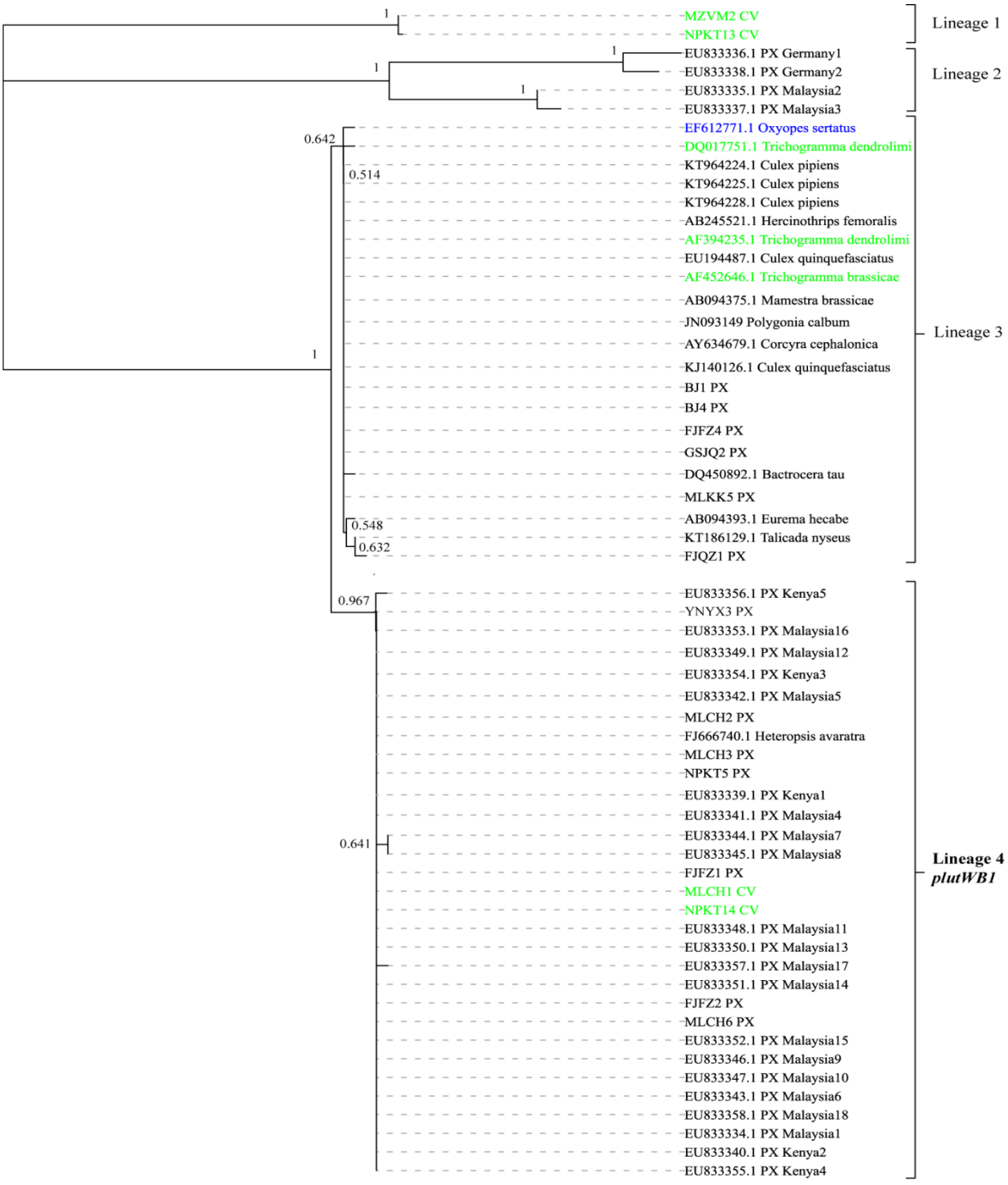


Figure 3.1 The *wsp*-based phylogenetic tree of *Wolbachia* using the neighbor-joining algorithm with 1000 bootstraps. Tree labels are colored according to different host species (black: herbivore; green: parasitoid; blue: predator); CV indicates *C. vestalis* and PX means *P. xylostella*; Branches with a bootstrap > 0.5 are shown.

Genetic diversity

A total of 1,621 bp DNA was obtained from concatenation of three *P. xylostella* mitochondrial genes (hereafter referred as p3m). From 323 *P. xylostella* individuals, 187 polymorphic loci and 212 haplotypes were identified, with 174 haplotypes represented by single individuals and 12 haplotypes were identified in multiple individuals of the same populations. The p3m-based calculation resulted in relatively high haplotype diversity with an average of 0.931, except for FJXM (0.524) and SXSL (0.607). Nucleotide diversity was low with an average of 0.329%, except FJQZ (0.606%), MLCH (0.906%) and NPKT (0.682%) (Table 3.3).

For *C. vestalis*, a total of 1,232 bp DNA was obtained from concatenation of three mitochondrial genes (hereafter referred as c3m). From 326 individuals, 43 polymorphic loci and 29 haplotypes were identified, with 19 haplotypes coming from single individuals and three haplotypes were identified in multiple individuals of the same populations. The c3m-based calculation revealed low haplotype diversity with an average of 0.415, except for GZGY (0.667) and YNYX (0.769), and low nucleotide diversity with an average of 0.172% was recorded, except for GZGY (0.431%) and YNYX (0.795%).

Table 3.3. Parameters of genetic diversity and demographic history of the *P. xylostella* and *C. vestalis* populations based on three mitochondrial genes

Population	n	L	S	h	Hd	π(%)	Tajima's *D*	Fu's *Fs*
XJSHZ	4|1	1621|1232	11|0	4|1	-|-	-|-	- | -	| -
JLCC	18|14	1621|1232	45|6	16|2	0.987|0.143	0.421|0.070	-1.976* | -1.959*	-7.859** | 2.207
LNSY	17|21	1621|1232	26|9	17|4	1.000|0.614	0.285|0.278	-1.613 | 1.258	-15.584*** |3.870
GSJQ	5|1	1621|1232	7|0	5|1	1.000|-	0.185|-	-0.747 |-	-2.238 | -
BJ	8|4	1621|1232	20|1	8|2	1.000|-	0.383|-	-1.036 | -	-3.319* | -
TJ	4|19	1621|1232	8|2	4|2	-|0.105	-|0.017	- | -1.511*	- | 0.021
NX	1|2	1621|1232	0|3	1|2	-|-	-|-	- | -	-| -
SDQD	18|13	1621|1232	47|7	13|3	0.928|0.564	0.410|0.237	-2.108* | 1.122	-3.216* | 3.671
HNZZ	20|20	1621|1232	49|5	17|4	0.984|0.284	0.462|0.041	-1.842* | -1.974**	-7.260** | -1.565
SXSL	8|2	1621|1232	6|0	3|1	0.607|-	0.115|-	-0.920 | -	1.412 | -
AHHF	8|4	1621|1232	17|0	8|1	1.000|-	0.322|-	-1.028 | -	-3.771* | -
SH	15|19	1621|1232	31|0	14|1	0.990|-	0.338|-	-1.790 | -	-8.319 ***| -
CQ	7|0	1621|	9|	4|	0.714|	0.170|	-1.319 |	0.495 |
HBWH	16|10	1621|1232	30|7	15|3	0.992|0.378	0.314|0.114	-1.807* | -1.839 *	-10.052 ***| 1.160
SCLZ	0|8	|1232	|2	|2	|0.250	|0.041	| -1.310	| 0.762
NPKT	7|17	1621|1232	34|5	7|5	1.000|0.426	0.682|0.056	-1.168 | -1.719*	-1.386 | -2.308
GZGY	17|10	1621|1232	47|25	16|5	0.993|0.667	0.470|0.431	-1.931*|-1.899**	-8.345*** | 1.728
FJFZ	13|19	1621|1232	17|6	12|2	0.987|0.199	0.210|0.097	-1.758 | -0.988	-8.828*** | 3.392
FJXM	7|14	1621|1232	3|6	3|2	0.524|0.363	0.065|0.177	-0.654 | 0.550	0.110 | 4.962*
FJPT	8|3	1621|1232	16|0	8|1	1.000|-	0.291|-	-1.213 | -	-4.09* | -
FJZZ	7|3	1621|1232	18|3	7|1	1.000|-	0.376|-	-0.952 |-	-2.550* | -
YNYX	15|13	1621|1232	43|23	14|6	0.990| 0.769	0.464|0.795	-1.903 * | 1.389	-6.435**| 3.524
FJQZ	11|10	1621|1232	36|7	8|3	0.945|0.378	0.606|0.114	-0.940 | -1.839*	0.216 |1.160
GDGZ	17|17	1621|1232	25|0	15|1	0.985|-	0.270|-	-1.736 | -	-9.920*** | -
GXNN	14|19	1621|1232	23|20	12|4	0.978|0.380	0.285|0.328	-1.528 | -1.126	-5.970** | 4.333*

Population	n	L	S	h	Hd	π(%)	Tajima's *D*	Fu's *Fs*
TLPH	7|10	1621|1232	15|0	6|1	0.952|-	0.294|-	-1.228 | -	-1.228 | -
VTDL	14|14	1621|1232	26|0	14|1	1.000|-	0.313|-	-1.613 | -	-10.580***| -
JXNC	14|14	1621|1232	24|4	12|4	0.978|0.396	0.283|0.046	-1.657 *| -1.798*	-5.995** | -1.640
MLKK	12|10	1621|1232	14|2	7|3	0.773|0.511	0.209|0.054	-1.143 | -0.184	-1.028 | -0.272
MLCH	11|13	1621|1232	35|1	7|2	0.909|0.282	0.906|0.023	0.912 | -0.274	-2.451 | 0.240
MZVM	0|2	|1232	|0	|1	|-	|-	|-	|-

Notes: n was the population size，L meant the length of DNA fragments, S indicated segregating sites, h and Hd indicate the number of the haplotypes and haplotype diversity, π is nucleotide diversity, "-" means the population with less than 5 individuals or with only one haplotype was not used for calculation of population parameters; "|" is the separation of *P. xylostella* and *C. vestalis*, the significance of statistic tests were indicated by "*" (P<0.05), "**" (P<0.01) and "***" (P<0.001).

Phylogenetic relationship

The analysis based on p3m revealed low genetic differentiation among individuals (Figure 3.2). No isolated clusters were formed by individuals from specific geographic regions or populations. A distinct clade of *P. xylostella* consisted of five individuals from different geographic locations and infected with *Wolbachia plutWB1* (Delgado and Cook 2009). Other individuals infected by *Wolbachia* were randomly distributed in the mitochondrial phylogeny (Figure 3.2).

The c3m-based phylogenetic tree indicated that the major *C. vestalis* cluster (Cluster 1) was formed by the samples from China, Malaysia, and Vietnam, while Cluster 2 derived from Chinese populations, including individuals from TLPH, NPKT, and MZVM. An outlying cluster (Cluster 3), composed of samples from northeast China, was derived from Cluster 2 (Figure 3.3). The *COI*-based global phylogeny was constructed using the *COI* gene sequences isolated in this study and additional sequences from India, Kenya, Benin, Hungary, Malaysia, New Zealand and Russia (Figure 3.4). These recruited individuals scattered within the previously defined clusters (Figure 3.5).

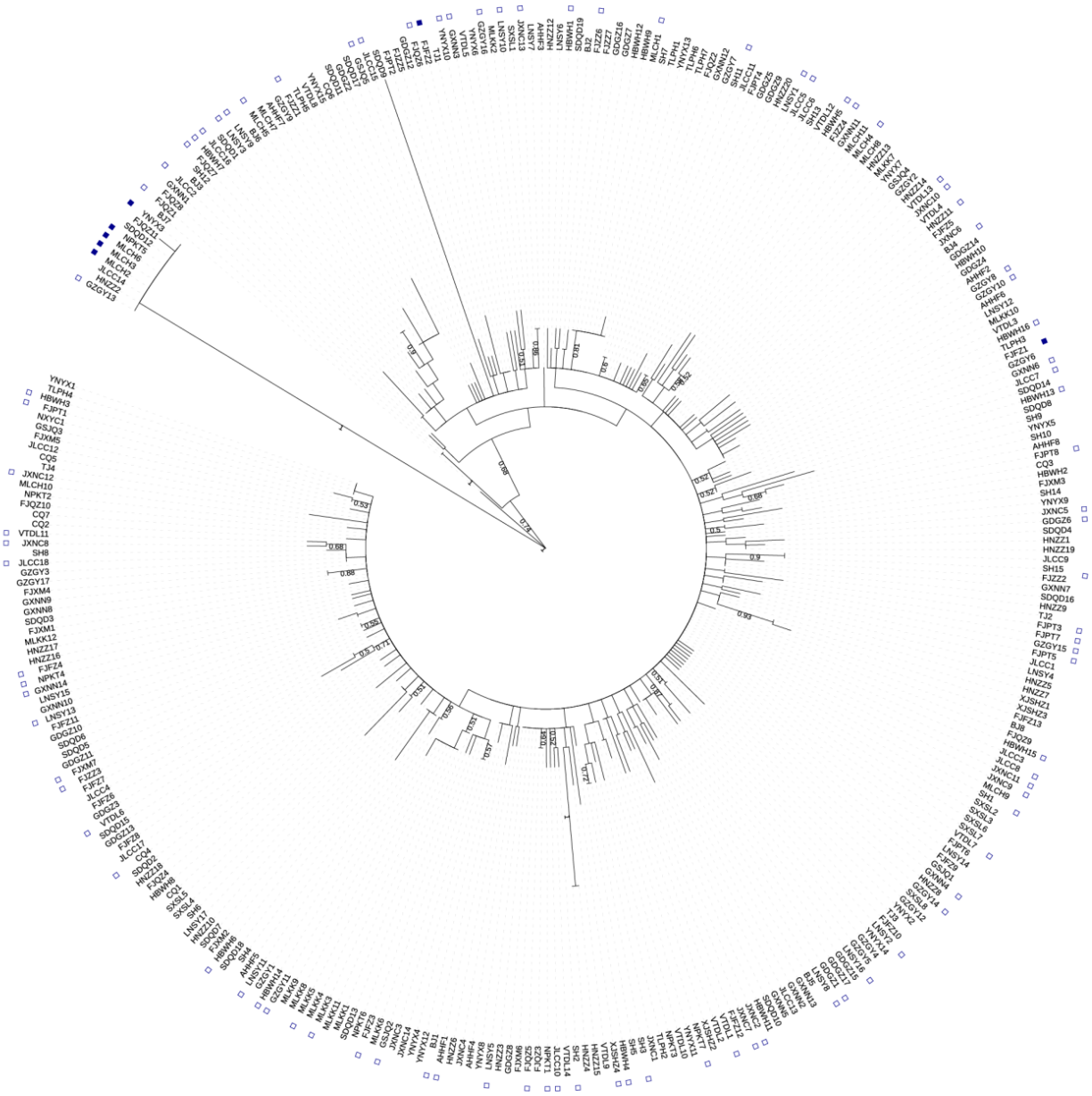


Figure 3.2 Phylogenetic tree of *P. xylostella* based on concatenated *COI*, *Cytb* and *NadhI* genes using maximum likelihood algorithm with 1000 bootstraps. Full diamonds indicate infection of *plutWB1*, and empty diamonds represent infection of other *Wolbachia* lineages. Branches with a bootstrap > 0.5 were showed.

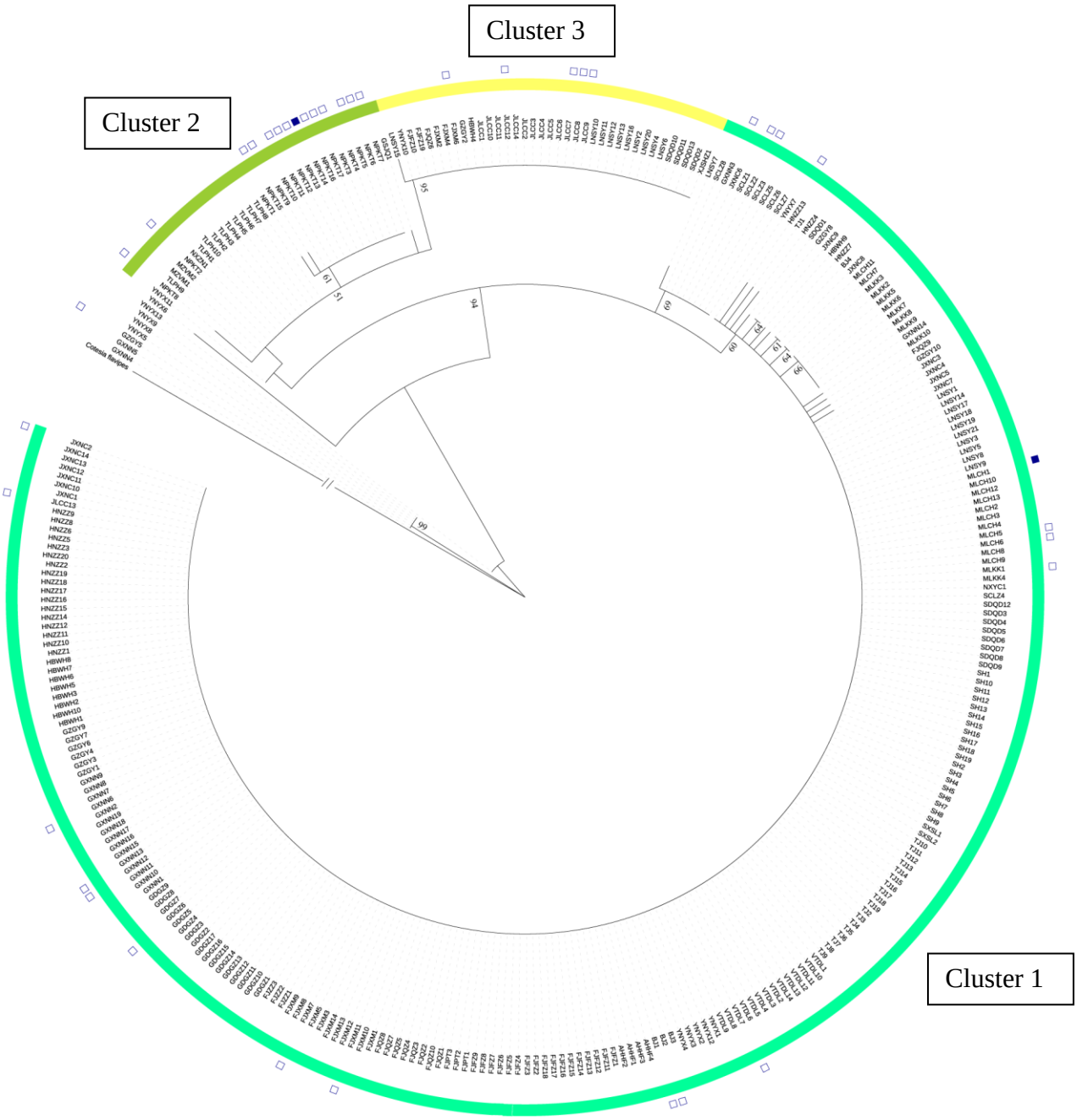


Figure 3.3 Phylogeny of *C. vestalis* based on concatenated *COI*, *Cytb* and *NadhI* genes using maximum likelihood algorithm with 1000 bootstraps. Full diamonds indicate the infection of *plutWB1*, and empty diamonds only denote the infection of *Wolbachia*. Branches with a bootstrap > 0.5 were indicated.

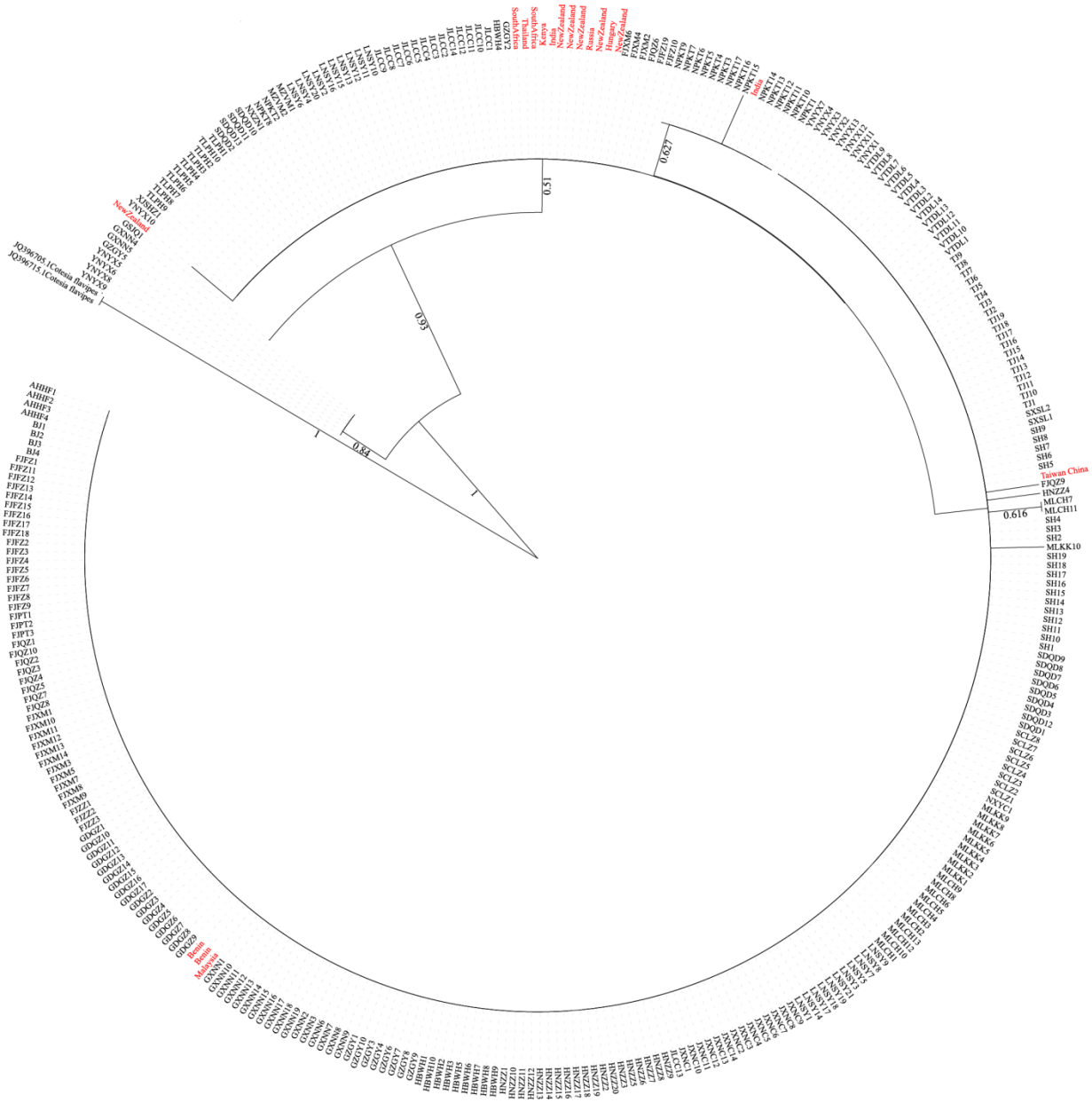


Figure 3.4 Phylogeny of global *C. vestalis* samples based on *COI* gene (545 bp) using maximum likelihood algorithm with 1000 bootstraps. Two *C. flavipes* individuals denoted outgroups. Individuals in red indicate recruited individuals from Europe, Africa, Oceania and Asia. Branches with > 0.5 supports were indicated.

Haplotype network

Analysis of a haplotype network was performed using the Cytb-based data for *P. xylostella* (Figure 3.5). The Cytb-based network showed a star-like shape with many unique haplotypes presented at the terminals indicating a recent population expansion event. In terms of such a Cytb-based network, the haplotype 2 (H2) was dominant and present in almost every sampled population (Figure 3.5).

For *C. vestalis*, in the c3m-based network, the dominating haplotype, haplotype 1 (H-1 in Cluster 1) was distributed in populations of East China as well as populations of Vietnam and Malaysia (Figure 3.6). Haplotypes identified in the populations TLPH, NPKT and MZVM (Cluster 2) are derived from haplotypes in Cluster 1 (Figure 3.6). Haplotypes in Cluster 3 are derived from Cluster 2 and were co-distributed with distinct haplotypes (Cluster 1) in the populations in China (Figure 3.6).

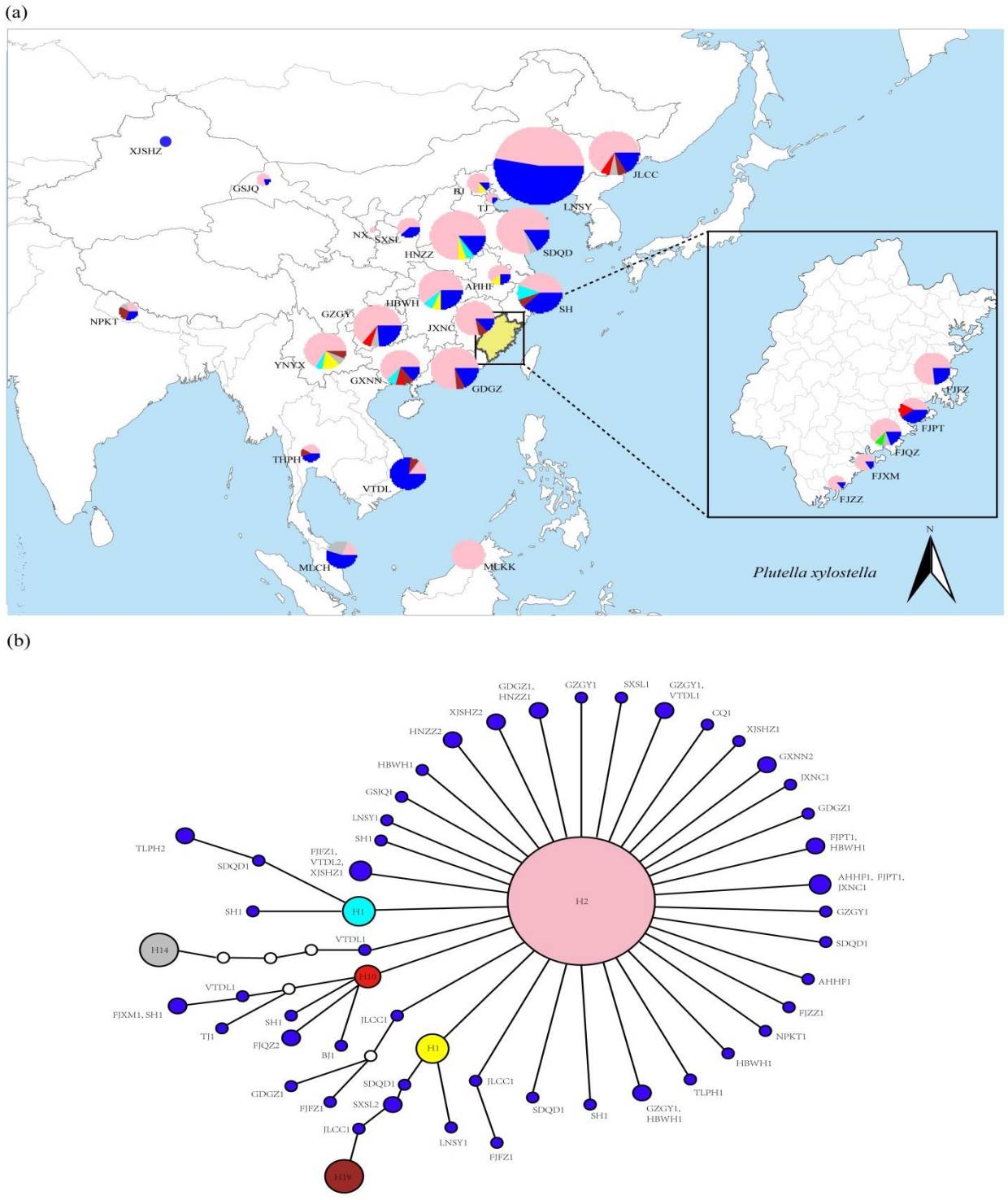


Figure 3.5 Haplotype distribution (a) and network (b) of *P. xylostella* based on *Cytb* gene across the sample locations. Haplotypes with a frequency ≤ 4 are illustrated with blue color and labeled with location names and numbers. Small empty circles represent unsampled individuals or missed haplotypes.

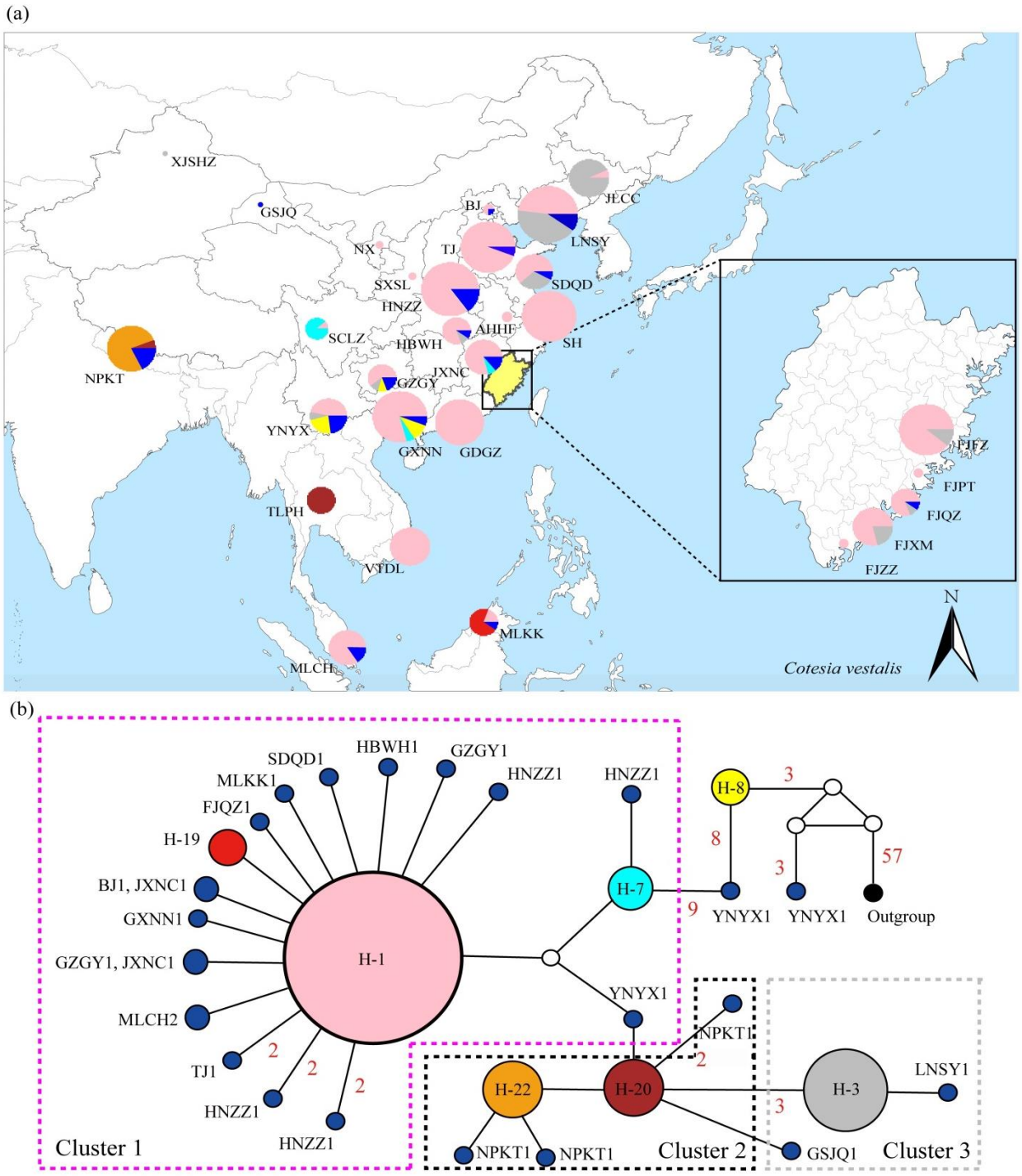


Figure 3.6 Haplotype distribution (a) and network (b) of *C. vestalis* based on concatenated *COI*, *Cytb* and *NadhI* genes (c3m) across the sample locations. Haplotypes with a frequency ≤ 4 are illustrated with blue color and labeled with location names and numbers. Small empty circles represent unsampled locations or missed haplotypes. The number of mutations >1 is shown next to branches.

Demographic history

Neutrality tests for *P. xylostella* were conducted using Tajima's *D* and Fu's *Fs* statistics (Table 3.3). The p3m-based Tajima's *D* and Fu's *F* statistics were significantly negative when considering all sampled populations as one group with Tajima's *D* = -2.501 (*P* < 0.001) and Fu's *Fs* = -5.532 (*P* < 0.05). Most of the populations had Tajima's D values not significantly different from zero, except in JLCC, SDQD, HNZZ, HBWH, GZGY, YNYX, and JXNC. When a significantly negative Tajima' D value is consistently associated with a significantly negative Fu's *Fs* value, recent population expansion is inferred. The p3m-based mismatch distributions were unimodal when considering all sampled individuals as one group (Figure 3.7), indicating a recent expansion of *P. xylostella* populations in East Asia.

Neutrality tests for *C. vestalis* showed that c3m-based Tajima's *D* and Fu's *Fs* statistics were significantly negative when considering all sampled populations as one group with Tajima's *D* = -1.747 (*P*< 0.05) and Fu's *Fs* = -10.475 (*P*< 0.001). As in *P. xylostella*, most *C. vestalis* populations showed Tajima's D values not significantly different from zero, except in JLCC, TJ, HNZZ, HBWH, NPKT, GZGY, JXNC, and FJQZ (Table 3.3). The Fu's *Fs* values were significantly negative in FJXM and GXNN. In the defined clusters, only Cluster 1 showed a significantly negative value of Tajima's *D* = -2.323 (P < 0.01) and large negative Fu's *Fs* = -23.426, *P*< 0.001), which indicated a recent population expansion event. No population expansion events could be inferred in Cluster 2 (Tajima's D= -1.421 and Fu's *Fs*= -3.066) or in Cluster 3 (Tajima's *D*= -1.133 and Fu's *Fs*= -1.362). The c3m-based mismatch distributions were multimodal when considering all sampled populations as one group, and all three defined clusters showed unimodal distributions (Figure 3.7).

The results of BEAST showed that the time to the most recent common ancestor (TMRCA) of *C. vestalis* expansion fell within the time gap formed by *P. xylostella* in the Old World and in Oceania, which indicated that *C. vestalis* could have expanded during the invasion of *P. xylostella* from Europe to Oceania (Figure 3.8).

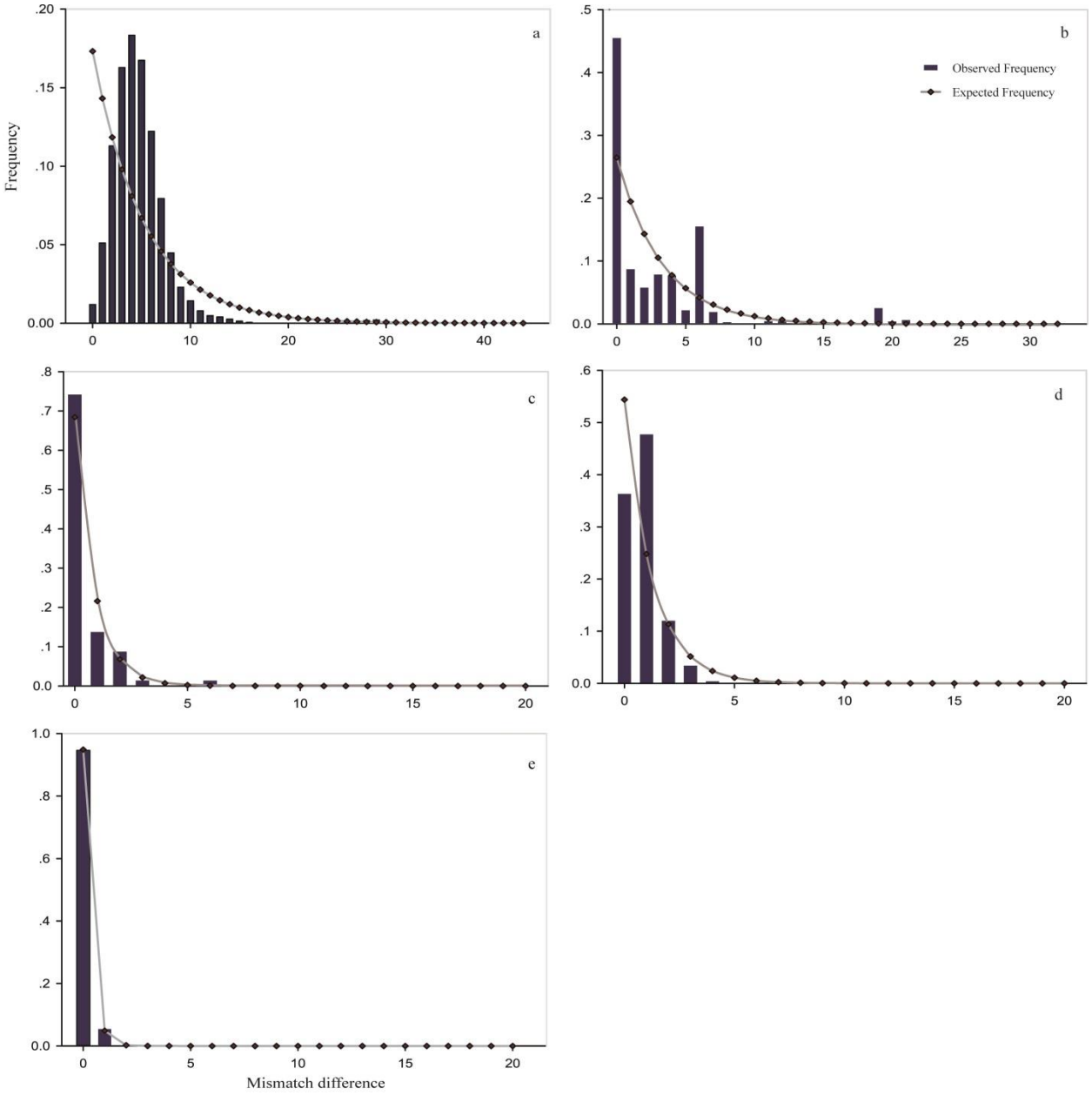


Figure 3.7 Mismatch distribution of *P. xylostella* and *C.vestalis* based on concatenated *COI*, *Cytb* and *NadhI* genes. a) *P. xylostella* (all individuals), b) *C.vestalis*(all individuals), c) Cluster 1 of *C.* v*estalis*, d) Cluster 2 of *C.* v*estalis*, and e) Cluster 3 of *C.* v*estalis*

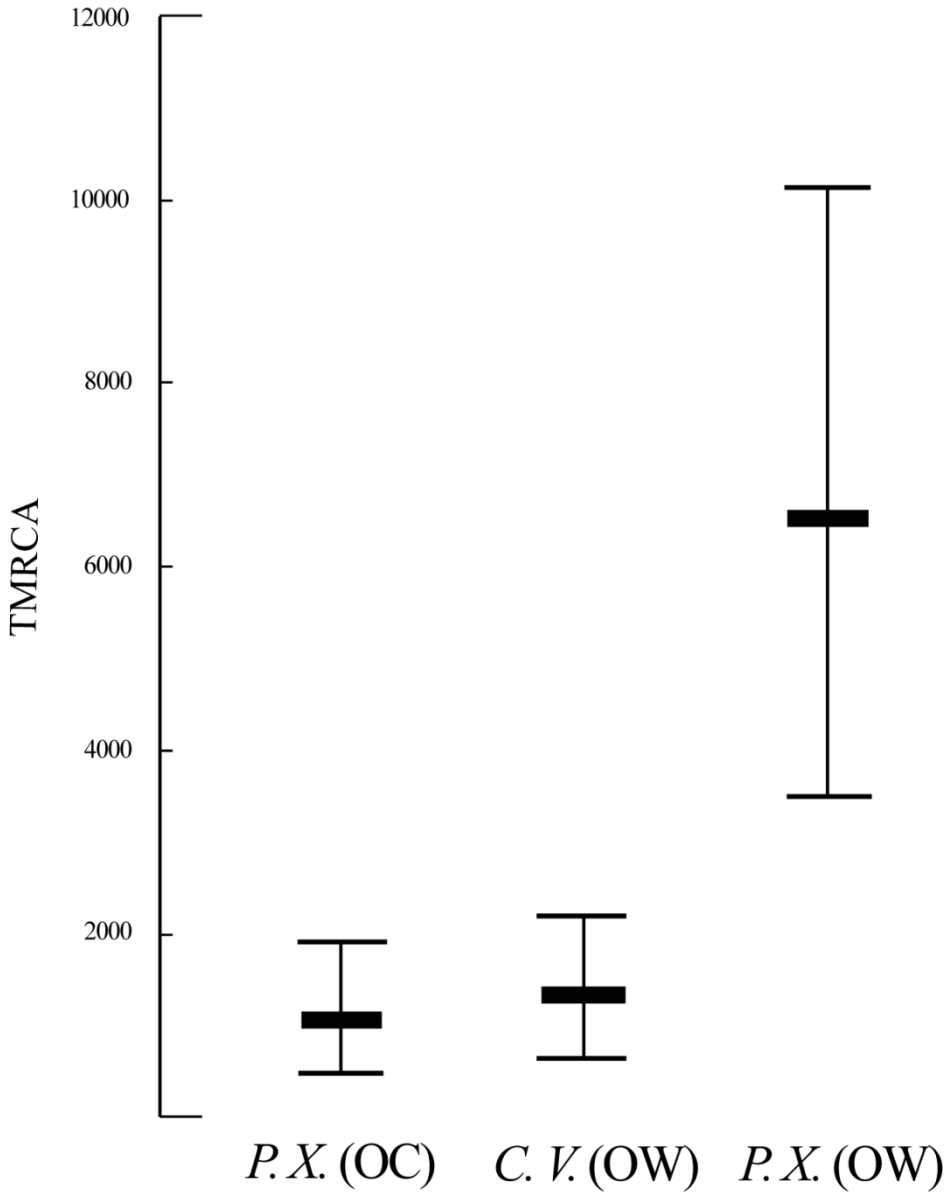


Figure 3.8 Divergence time estimates were based on the *COI* gene of *P. xylostella* and *C.vestalis*. P.X. and C.V. stand for *Plutella xylostella* and *Cotesia vestalis*, OC, OW indicate Oceania and Old World, respectively.

3.**4. Discussion**

In this study, we used an interactive system involving an invasive herbivore, *P. xylostella,* and its parasitoid, *C. vestalis*, and demonstrated a case where the genetic conponents of a higher trophic level (*C. vestalis*) in the local community was influenced by the invasion of an alien host herbivore (*P. xylostella*). As a consequence of ecological sorting, this parasitoid transferred from an original (unknown) host to *P. xylostella* and underwent significant anthropogenic population expansion. In addition, the endosymbiont *plutWB1* in *P. xylosetlla* was also introduced into local arthropod communities in the course of invasion. We also present data suggesting that the invasion by an alien species triggered significant evolutionary (distribution) changes in its newly associated parasitoid and led to the formation of a new biological interaction.

Two major schools of thought are proposed regarding how a set of geographically widespread species come to occur together. The first one assumes long-term and stable interactions, dominated by co-evolution and host tracking, leading to parallel diversification of hosts and their enemies (Schluter 2000). The second one is ecological sorting (Weiher and Keddy 2001), allowing species from higher trophic levels (e.g. parasitoids) to switch among different but ecologically similar sets of hosts (Nicholls et al. 2010). The host-tracking hypothesis emphasizes that the distribution shifts of parasites follows the trend of their host species either synchronously or with a temporal delay by showing phylogenetic concordance between such two closely interacting species (Schluter 2000). Under the ecological sorting model, discordance of phylogenies between interacting species is expected in the case of host switching among ecologically similar but unrelated hosts (Althoff 2008; Nicholls, et al. 2010).

C. vestalis, native in East and Southeast Asia (Ooi 1992; Alvi and Momoi 1994; Liu et al. 2000), is one of the most common parasitic wasps of *P. xylostella*. Previously *C. vestalis* was inferred to originate from Europe as the species was described from Ukrainian specimens (Delvare et al. 2004). However, our phylogenetic evidence (c3m- and *CO I*-based) suggests that *C. vestalis* populations in Europe, Africa, and Oceania are all derived from East Asia, and Southwest China is suggested to be the geographic origin of the examined *C. vestalis* samples based on phylogenetic evidence. This inference is further supported by c3m-based polymorphism data that

the *C. vestalis* populations YNYX and GZGY (both from Southwest China) possessed the highest genetic diversity, comparable with previous studies on insect species (Savolainen et al. 2002; Ma et al. 2012). Although no samples from the New World were included in this study, it is reasonable to speculate that haplotypes in the New World are derived from haplotypes of the Old World as *C. vestalis* was reported to be recently introduced into North America (Shelton 2004) and South America (e.g. Brazil) (Delvare et al. 2004) as a biocontrol agent.

Our comprehensive analysis implied regional expansions of the examined *C. vestalis* populations. Driven by the invasion of *P. xylostella* into our study areas, the population expansions of C. *vestalis* started from Southwest China, and diffused towards other surrounding courtiers following the human-aided dispersal of *P. xylostella* from the Old world to Oceania (Endersby et al. 2006; Pichon et al. 2006; Saw et al. 2006). Such an expansion was possibly a case of ecological sorting that enabled *C. vestalis* to shift from its original host to *P. xylostella*, which is abundant in intensive *Brassica* vegetable farming systems (Talekar 2004). Low genetic diversity identified in our *C. vestalis* populations suggests a facilitation of its range expansion during the course of *P. xylostella* invasion. A comparable observation has also been reported for two *Diadegma* parasitoids of *P. xylostella* in Europe (Juric et al. 2016).

Biological invasion may also facilitate the transmission of exotic bacteria to local communities (Stobbs et al. 1992; Pimentel et al. 2001; Sachs and Malaney 2002; Pimentel 2011), and affect the genetic makeup of local species pools. Major influences of *Wolbachia* on the mitochondrial genome include reducing the mitochondrial genetic diversity and shaping the maternal genetic divergence in host populations (Hurst and Jiggins 2005; Opijnen et al. 2005; Oliveira et al. 2008; Raychoudhury et al. 2010). In this study, we observed a distinct maternal lineage in *P. xylostella* (individuals from Nepal, Malaysia and China) infected with *Wolbachia plutWB1*. This lineage of *plutWB1* was reported to infect individuals in Kenya and Malaysia, and formed a distinct maternal lineage including only *plutWB1*-infected individuals (Delgado and Cook 2009), which is consistent with our findings. We also found evidence of horizontal transfer of *plutWB1* from *P. xylostella* to *C. vestalis* by showing that two *C. vestalis* individuals from Nepal and Malaysia were infected by *plutWB1*, while not forming a distinct maternal lineage. In addition, one specific *Wolbachia* lineage (lineage 3) was identified in many insect species including herbivores,

parasitoids and one predator. Parasitoids directly kill or sterilize their hosts and are not able to contribute to the horizontal transfer of *Wolbachia* between different host species, so the presence of such a *Wolbachia* lineage in various herbivores is likely from food intake. This hypothesis is supported by a recent study of wild megachilid bees, in which the plants can act as hubs for bacterial transmission between multiple organisms (McFrederick et al. 2016).

Species may change life history strategies, distribution, habitat associations, and trophic interactions, in the context of climate change (Menéndez, et al. 2014; Schmitz and Barton 2014). Range shifts of species, including expanding to higher elevations and latitudes in response to global warming (in accordance with the level of warming in specific regions), have been documented (Menéndez, et al. 2014). According to 11 years' light trapping data across the Bohai Gulf (Fu et al. 2014), *P. xylostella* can undergo annual dispersals from southern China (year-round persistence regions) to northern China (seasonal persistence regions). Similar northward annual migrations have been observed for North American (Dosdall et al. 2001) and European populations as well (Chapman et al. 2002), and such a trend is expected to be observed more frequently or in wider seasonal persistence regions in the context of growing temperature. With strong environmental adaptability and mobility, it is reasonable to predict that *P. xylostella* will continue to expand its range with a long-term trend of global warming (Li et al. 2015). Parasitoids such as *C. vestalis*, with global distribution and high adaptive capacity in various environments (especially tolerance to high temperature), are thus expected to play increasingly significant roles in control of this pest species. However, such a comparative advantage of *C. vestalis* might be gradually weakened if the downward trend of genetic and haplotype diversity identified in this study can't be suppressed (Lommen et al. 2016).

Chapter 4 Genetic variability provides insight into geographic patterns and strong adaptation of *Plutella xylostella*

4.1 Introduction

The diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), is one of the most destructive pests of economically important food crops, including rapeseed, cauliflower and cabbage. A recent estimate of the total costs associated with the DBM damage and management worldwide was US$4 - 5 billion per annum (Zalucki et al., 2012).

P. xylostella is not inherently adapted for long-distance migration, however, it can disperse over wide spatial scales under favourable conditions, e.g. via the jetstream (Furlong et al., 2013). Increased productions of cruciferous crops, insecticide intensification, as well as trade globalization over the past decades have exacerbated the worldwide pest status of *P. xylostella*. This insect is the first agricultural pest to have evolved field resistance to dichlorodiphenyltrichloroethane (DDT) in the 1950s (Ankersmit, 1953) and to *Bacillus thuringiensis* (Bt) toxins in the 1990s (Heckel et al., 1999) and has developed resistance to all classes of insecticide, making it extremely difficult to control (Furlong et al., 2013). However, knowledge of its global patterns of genetic variation and contemparoty distribution remains surprisingly inadequate, and limits further understanding of genetic mechanisms of adaptation to different climates and resistance to agrochemicals.

P. xylostella has the most extensive distribution of all Lepidoptera (Talekar and Shelton, 1993). Conflicting hypotheses have been proposed for the ancestral origin of this species, and little is known about its global distribution and migration. Aiming to improve annual migration monitoring and biocontrol-based integrated management of *P. xylostella*, we sought to use population genomics to identify the ancestral origin, dispersal patterns and formation of different lineages of *P. xylostella*, with a view to the contribution of evolutionary events, natural and anthropogenic factors to contemporary distribution and genetic makeup of *P. xylostella* populations in the Americas. To do so, we sequenced the genomes of 177 *P. xylostella* sampled from 12 countries across the Americas (Figure 4.1; Table 4.1; Table 4.2) and analyzed these using the *P. xylostella* reference genome sequence (You et al., 2013).

Additionally, DBM populations from different locations differ in their level of susceptibility to insecticides suggesting possible genetic differences and adaptive capacity to respond to various selection pressures (Pichon et al. 2006). Therefore, how genetic polymorphism contributes to its rapid adaptation to insecticides is also one of the key questions that is addressed in this chapter. The genome-wide single-nucleotide polymorphism (SNP)-based results should enrich our insights into underlying mechanisms of insecticide resistance in such a notorious pest from a geographically diverse collection, which is ranked second in the Arthropod Pesticide Resistance Database (APRD) for the highest number of insecticides with reported resistance (APRD 2012).

4.2 Materials and methods

Samples and DNA extraction

A total of 174 *P. xylostella* individuals were collected during 2012-2014 from 38 geographical locations (sampling sites) in 12 countries across the Americas (Figure 4.1; Table 4.1). Larvae and pupae were randomly collected from cruciferous vegetable fields in each location. *P. xylostella* is a global pest that can be found wherever crucifers are grown and is believed to be the most universally distributed of all Lepidoptera (Talekar and Shelton, 1993; Furlong et al., 2013). The samples that we collected cover broad regions from year-round persistence to seasonably suitable for growth and development of *P. xylostella* throughout the Americas, with 12 field samples from South America (SA, n = 55 individuals) and 26 field samples from North America (NA, n = 119 individuals) (Table 4.2). Samples were morphologically checked to confirm their identity, and preserved in 95% alcohol at -80ºC in freezers prior to DNA extraction.

Field-collected samples were individually washed twice using double-distilled water, and then dissected to remove the midgut and any endoparasitoids to exclude potential DNA contamination. DNA was extracted for each of the individuals using DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA was eluted from the DNeasy Mini spin column in 200µl TE buffer.

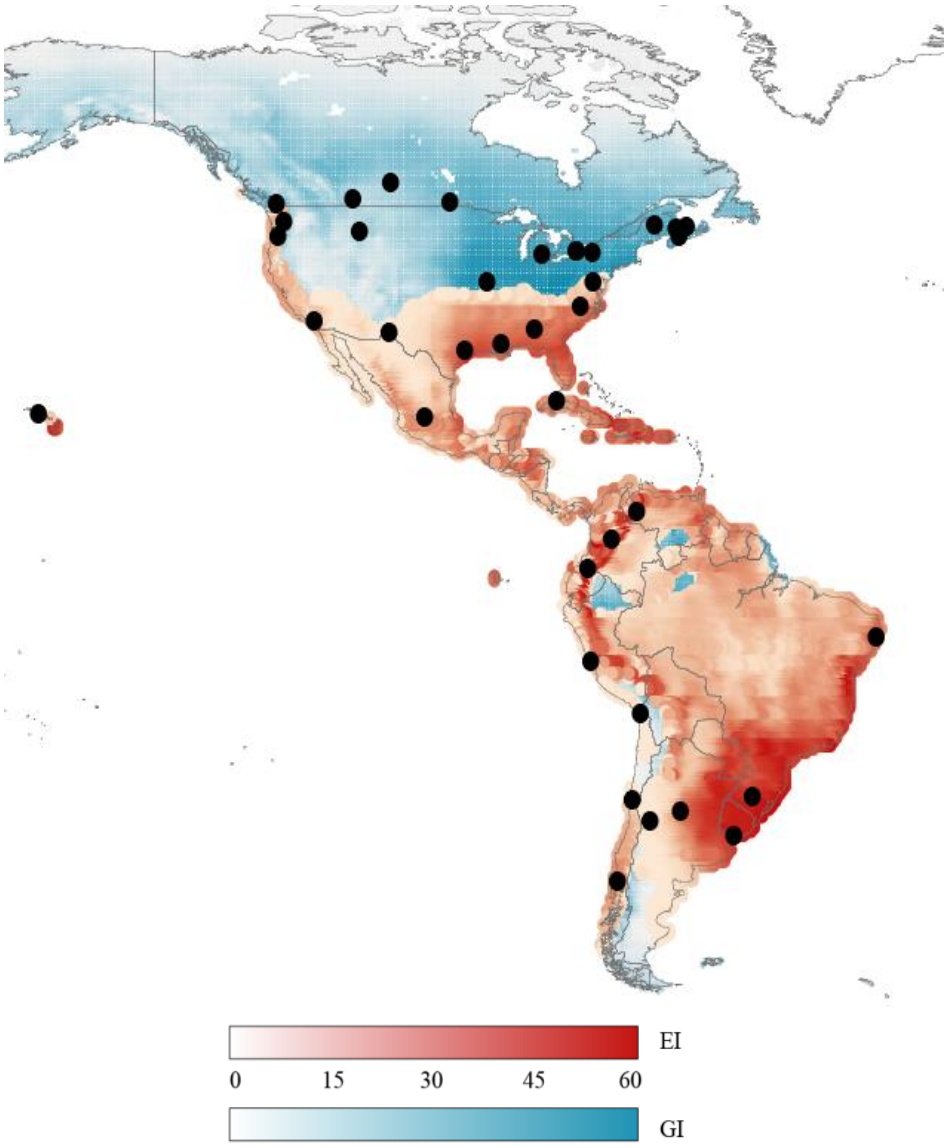


Figure 4.1 Locations of the *P. xylostella* samples used in this study. Areas shaded in red show regions with positive eco-climatic index (EI) values where *P. xylostella* can persist year-round. Areas shaded in blue show regions with a positive growth index (GI) and EI being zero where *P. xylostella* cannot persist year-round but can become a seasonal pest following immigration of moths from elsewhere.

Table 4.1 Sample information

Population ID	Location	GPS	Sampling date
Pop1_NA	Prince Edward Island, Canada	N46.39, W63.29	30-Aug-2012
Pop2_NA	Nova Scotia, Canada	N45.12, W64.44	30-Aug-2012.
Pop3_NA	Quebec, Canada	N43.14, W79.47	15-Dec-2012
Pop4_NA	Manitoba, Canada	N49.48, W97.93	17-July-2013
Pop5_NA	Saskatchewan, Canada	N52.15, W106.57	14-Aug-2013
Pop6_NA	Vauxhaul, Alberta, Canada	N50.09, W112.12	31-Jul-2013
Pop7_NA	Hawaii, USA	N21.42, W158.02	3-Mar-2013
Pop8_NA	North Carolina, USA	N35.60, W78.85	24-Jul-2013
Pop9_NA	Montana, USA	N45.72, W111.15	11-Sep-2013
Pop10_NA	Maine, USA	N46.64, W68.01	30-Aug-2013
Pop12_NA	Michigan, USA	N42.70, W84.49	12-Sep-2013
Pop13_NA	Missouri, USA	N38.85, W92.43	14-Sep-2013
Pop14_NA	Maryland, USA	N39.01, W76.93	17-Sep-2013
Pop15_NA	Alabama, USA	N32.57, W85.50	21-Sep-2013
Pop17_NA	Texas, USA	N29.74, W95.73	28-Sep-2013
Pop18_NA	Louisiana, USA	N30.60, W90.37	1-Oct-2013
Pop19_NA	New Mexico, USA	N32.27, W106.75	3-Oct-2013
Pop20_NA	Oregon state, USA	N45.07, W123.02	6-Sep-2013
Pop22_NA	California state, USA	N33.68, W117.78	7-Oct-2013
Pop23_NA_C	Vancouver, Canada	N49.25, W123.24	30-Jul-2013
Pop23_NA_A	New York, USA	N42.87, W77.08	9-Sep-2013
Pop24_NA	Seattle, USA	N47.06, W122.19	23-Sep-2013
Pop26_NA	Ontario, Canada	N43.13, W79.31	3-Mar-2014
Pop27_NA	New Bruswick, Canada	N46.09, W64.79	15-Mar-2014
Pop28_NA	Havana, Cuba	N23.16, W82.29	15-Jan-2014
NA_M	Romita, Mexico	N20.88, W101.54	25-Mar-2014
Pop1_SA	Recife, Brazil	S8.26, W35.51	23-Mar-2013
Pop2_SA	Santa Maria, Brazil	S29.67, W53.69	27-Mar-2013
Pop3_SA	Montevideo, Uruguay	S34.84, W56.34	2-Apr-2013
Pop4_SA	Mendoza, Argentina	S32.92, W68.63	12-Apr-2013
Pop5_SA	Cordoba, Argentina	S31.52, W64.18	10-Apr-2013
Pop6_SA	Arica, Chile	S18.57, W70.06	29-Apr-2013

Population ID	Location	GPS	Sampling date
Pop7_SA	La Serena, Chile	S30.01, W71.25	25-Apr-2013
Pop8_SA	Osorno, Chile	S40.92, W73.36	18-Apr-2013
Pop9_SA	Huaral, Peru	S11.61, W77.24	8-May-2013
Pop10_SA	Tulcan, Ecuador	N0.79, W77.70	17-May-2013
Pop11_SA	Bogota, Colombia	N4.69, W74.22	29-May-2013
Pop12_SA	Barinas, Venezuela	N8.44, W70.56	24-Oct-2012

Table 4.2 Sequencing statistics

Sample	Sequencing data			Statistics of effective data		
	Raw (Mb)	Clean (Mb)	Mapped (Mb)	Mapped (%)	Coverage (%)	Depth (X)
POP1_NA_22	3597.05	3239.11	2935.57	90.63	75.96	10.28
POP1_NA_4	3490.41	3054.94	2602.4	85.19	75.77	9.14
POP1_SA_1	3441.99	3156.06	2878.53	91.21	75.41	10.15
POP1_SA_4	3542.48	3244.08	2865.39	88.33	75.2	10.13
POP1_SA_6	6228.37	5759.68	5093.18	88.43	77.8	17.41
POP1_SA_7	3719.34	3394.21	3032.42	89.34	75.51	10.68
POP1_SA_9	3938.9	3591.67	3283.83	91.43	75.89	11.51
POP10_NA_1	3995.24	3557.8	3166.15	88.99	76.34	11.03
POP10_NA_10	3579.56	3249.72	2913.69	89.66	76.71	10.1
POP10_NA_12	4711.49	4167.79	3793.05	91.01	76.97	13.11
POP10_NA_8	4108.64	3704.83	3296.92	88.99	77.09	11.37
POP10_NA_9	4104.03	3669.35	3265.55	89	77	11.28
POP10_SA_10	3939.15	3598.95	3256.6	90.49	76.57	11.31
POP10_SA_11	4056.25	3670.95	3323.16	90.53	77.2	11.45
POP10_SA_2	3604.46	3318.29	3020.98	91.04	76.37	10.52
POP10_SA_4	3764.77	3385.3	3076.68	90.88	76.55	10.69
POP10_SA_5	3612.66	3257.37	2966.44	91.07	76.08	10.37
POP11_SA_1	2431.05	2198.4	1851.55	84.22	73.45	6.7
POP11_SA_4	2671.38	2402.82	2188.99	91.1	74.24	7.84
POP11_SA_7	3418.25	3121.08	2758.35	88.38	75.86	9.67
POP11_SA_8	2775.46	2354.47	2132.71	90.58	73.86	7.68
POP12_NA_1	4005.56	3598.89	3247.6	90.24	76.39	11.31
POP12_NA_2	2304.65	2031.68	1811.21	89.15	72.5	6.64
POP12_NA_3	2865.37	2527.12	2168.23	85.8	74.05	7.79
POP12_NA_4	3639.56	3235.22	2935.6	90.74	75.88	10.29
POP12_NA_5	3196.08	2838.52	2543.45	89.6	74.76	9.05
POP12_SA_1	3352.7	3012.33	2742.51	91.04	75.91	9.61
POP12_SA_2	3329.98	3051.64	2778.63	91.05	75.45	9.79
POP12_SA_3	3525.01	3128.05	2864.32	91.57	75.77	10.05
POP12_SA_7	3168.55	2880.44	2594.83	90.08	76.04	9.08
POP13_NA_1	3811.67	3423.28	3086.86	90.17	76.51	10.73

Sample	Sequencing data			Statistics of effective data		
	Raw (Mb)	Clean (Mb)	Mapped (Mb)	Mapped (%)	Coverage (%)	Depth (X)
POP13_NA_5	3182.76	2841.19	2568.67	90.41	75.36	9.07
POP13_NA_6	3943.16	3589.32	3256.44	90.73	76.6	11.31
POP13_NA_7	3061.14	2719.42	2468.4	90.77	75.43	8.7
POP14_NA_1	3723.97	3283.46	2980.42	90.77	76.46	10.37
POP14_NA_12	4180.48	3789.53	3456.34	91.21	77.11	11.92
POP14_NA_13	3070.03	2723.31	2394.9	87.94	74.68	8.53
POP14_NA_15	3227.45	2819.07	2554.94	90.63	75.92	8.95
POP14_NA_2	4033.38	3570.56	2920.87	81.8	76.25	10.19
POP15_NA_2	3353.5	2983.3	2704.81	90.66	75.83	9.49
POP15_NA_3	4652.19	4150.3	3760.13	90.6	77.32	12.93
POP15_NA_4	3751.86	3364.03	3054.73	90.81	76.76	10.58
POP15_NA_5	2528.56	2264.61	1986.73	87.73	73.92	7.15
POP15_NA_6	4431.3	3930.32	3459.64	88.02	77.11	11.93
POP17_NA_1	3928.91	3456.7	3131.4	90.59	76.44	10.9
POP17_NA_3	4295.99	3821.57	3451.81	90.32	77.18	11.9
POP17_NA_4	4245.8	3788.69	3441.07	90.82	77.34	11.83
POP17_NA_5	4319.7	3901.09	3528.53	90.45	77.17	12.16
POP17_NA_6	5145.9	4609.55	4011.39	87.02	77.74	13.72
POP18_NA_1	2901.19	2644.3	2372.84	89.73	74.86	8.43
POP18_NA_21	2982.68	2693.42	2442.7	90.69	75.03	8.66
POP18_NA_22	3232.77	2910.12	2627.06	90.27	75.42	9.26
POP18_NA_23	4080.59	3673.63	3337.73	90.86	76.57	11.59
POP18_NA_4	2662.99	2409.89	2098.09	87.06	74.08	7.53
POP19_NA_1	3973.57	3579.4	3251.01	90.83	76.98	11.23
POP19_NA_10	4079.51	3664.35	3334.74	91.01	77.05	11.51
POP19_NA_11	3589.92	3225.17	2547.18	78.98	75.52	8.97
POP19_NA_5	3309.85	2978.94	2704.81	90.8	75.84	9.49
POP19_NA_9	4791.45	4356.82	3962.99	90.96	77.54	13.59
POP2_NA_26	3370.47	3051.66	2771.62	90.82	75.53	9.76
POP2_SA_12	3905.55	3561.65	3243.18	91.06	76.14	11.33
POP2_SA_2	3189.87	2902.56	2629.76	90.6	74.82	9.35
POP2_SA_24	3594.17	3316.2	3004.4	90.6	75.8	10.54
POP2_SA_25	3249.56	2955.01	2663.32	90.13	74.91	9.46

Sample	Sequencing data			Statistics of effective data		
	Raw (Mb)	Clean (Mb)	Mapped (Mb)	Mapped (%)	Coverage (%)	Depth (X)
POP2_SA_7	3307.4	3002.27	2725.17	90.77	74.36	9.75
POP20_NA_1	2607.55	2364.64	2149.25	90.89	74.19	7.7
POP20_NA_25	3362.34	3059.5	2779.19	90.84	75.49	9.79
POP20_NA_3	2714.8	2481.78	2254.57	90.84	74.36	8.06
POP20_NA_5	2967.15	2716.13	2470.13	90.94	75.02	8.76
POP22_NA_10	3711.28	3356.34	3058.04	91.11	76.61	10.62
POP22_NA_12	3566.34	3241.59	2943.86	90.82	76.19	10.28
POP22_NA_3	4073.38	3618.85	3267.2	90.28	76.85	11.31
POP22_NA_4	3727.12	3298.38	3009.75	91.25	76.16	10.51
POP23A_NA_1	2910.6	2566.16	1901.47	74.1	73.87	6.85
POP23A_NA_10	3287.24	2985.88	2709.87	90.76	75.61	9.53
POP23A_NA_3	2727.96	2411.11	2181.59	90.48	74.73	7.76
POP23A_NA_4	3654.73	3207.99	2686.66	83.75	75.94	9.41
POP23A_NA_6-2	2798.87	2485.23	2260.33	90.95	73.74	8.15
POP23C_NA_1	2505.26	2251.99	2044.62	90.79	73.88	7.36
POP23C_NA_10	3823.96	3329.37	2909.24	87.38	75.96	10.19
POP23C_NA_5	2826.86	2428.33	2200.27	90.61	74.25	7.88
POP23C_NA_6	3381.68	3014.57	2724.32	90.37	75.62	9.58
POP23C_NA_8	2128.52	1948.52	1765.74	90.62	73.06	6.43
POP24_NA_1	3496.34	2972.42	2687.06	90.4	75.38	9.48
POP24_NA_10	4447.53	3967.66	3601.27	90.77	77.32	12.39
POP24_NA_2	4735.12	4216.32	3482.06	82.59	76.76	12.07
POP24_NA_3	4546.8	3966.03	3578.05	90.22	76.99	12.36
POP24_NA_5	4121.58	3719.26	3229.49	86.83	76.91	11.17
POP26_NA_1	3189.87	2846.98	2583.27	90.74	74.86	9.18
POP26_NA_11	5747.18	5130.76	4702.46	91.65	77.54	16.13
POP26_NA_12	4396.44	3659.59	3335.6	91.15	76.7	11.57
POP26_NA_2	2616.59	2304.46	2094.55	90.89	73.1	7.62
POP26_NA_3	2319.77	2026.13	1833.92	90.51	72.88	6.69
POP26_NA_4	2676.74	2371.89	2155.37	90.87	73.81	7.77
POP26_NA_5	3520.77	2976.9	2715.97	91.23	75.73	9.54
POP26_NA_5	3372.6	3055.47	2778.6	90.94	75.52	9.79
POP26_NA_6	3578.51	3018.14	2753.46	91.23	75.75	9.67

Sample	Sequencing data			Statistics of effective data		
	Raw (Mb)	Clean (Mb)	Mapped (Mb)	Mapped (%)	Coverage (%)	Depth (X)
POP26_NA_8	3368.25	2828.61	2579.4	91.19	75.4	9.1
POP27_NA_2	2664.53	2430.06	2212.56	91.05	74.4	7.91
POP27_NA_3	3890.53	3519.5	3196.73	90.83	76.37	11.13
POP27_NA_4	3008.24	2753.42	2502.81	90.9	74.26	8.96
POP27_NA_5	3013.99	2774.26	2516.51	90.71	74.94	8.93
POP27_NA_6	3028.87	2786.34	2527.69	90.72	75.3	8.93
POP28_NA_2	2723.25	2434.12	2218.45	91.14	74.3	7.94
POP28_NA_4	2743.85	2399.84	2186.19	91.1	73.93	7.87
POP28_NA_8	3361.32	2988.35	2724.96	91.19	75.79	9.56
POP3_NA_1	3537.06	3211.62	2923.66	91.03	74.32	10.46
POP3_NA_3	3202.59	2912.84	2647.73	90.9	73.81	9.54
POP3_NA_4	3624.16	3249.72	2958.21	91.03	74.84	10.51
POP3_NA_6	4233.12	3803.13	3461.93	91.03	75.75	12.16
POP3_NA_7	4103.95	3667.42	3335.37	90.95	75.52	11.75
POP3_SA_3	4385.39	3955.74	3595.06	90.88	76.67	12.47
POP3_SA_4	4002.25	3663.42	3270.07	89.26	76.31	11.4
POP3_SA_5	4071.52	3655.21	3315.38	90.7	76.43	11.54
POP3_SA_7	4325.49	3904.89	3400.28	87.08	76.53	11.82
POP3_SA_8	3986.44	3569.51	3241.05	90.8	76.39	11.29
POP4_NA_1	4070.24	3563.96	3200.77	89.81	76.87	11.08
POP4_NA_21	3007.41	2736.7	2459.44	89.87	74.59	8.77
POP4_NA_5	3481.34	3172.11	2876.5	90.68	76.15	10.05
POP4_NA_6	3060.98	2772.59	2513.23	90.65	75.38	8.87
POP4_SA_11	3052.3	2759.96	2509.23	90.92	74.72	8.93
POP4_SA_29	3573.22	3253.09	2955.19	90.84	75.63	10.39
POP4_SA_37	3816.53	3468.27	3133.27	90.34	75.8	10.99
POP4_SA_39	3348.27	3035.21	2757.44	90.85	75.24	9.75
POP4_SA_42	2679.98	2454.61	2231.7	90.92	74.17	8
POP5_NA_4	2739.39	2527.4	2296.22	90.85	74.84	8.16
POP5_NA_5	2627.38	2423.83	2207.36	91.07	74.78	7.85
POP5_NA_6	5302.72	4571.8	4160.93	91.01	77.88	14.21
POP5_NA_7	2884.72	2647.21	2404.95	90.85	75.29	8.5
POP5_NA_8	2925.3	2694.16	2449.91	90.93	75.47	8.63

Sample	Sequencing data			Statistics of effective data		
	Raw (Mb)	Clean (Mb)	Mapped (Mb)	Mapped (%)	Coverage (%)	Depth (X)
POP5_SA_3	2911.04	2659.04	2419.23	90.98	74.57	8.63
POP5_SA_5	4110.16	3776.46	3433.22	90.91	76.84	11.88
POP5_SA_6	3253.2	2968.49	2670.5	89.96	74.99	9.47
POP5_SA_6-2	4046.71	3665.3	3323.56	90.68	76.77	11.52
POP5_SA_8	4055.24	3650.37	3322.07	91.01	76.46	11.56
POP6_NA_1	3638.41	3340.66	2404.94	71.99	74.94	8.54
POP6_NA_11	4145.08	3817.19	3346.08	87.66	76.92	11.57
POP6_NA_12	3125.64	2703.48	2454.75	90.8	74.98	8.71
POP6_NA_5	3099.11	2833.72	2571.82	90.76	75.57	9.05
POP6_NA_6	3334.22	3062.14	2734.16	89.29	75.74	9.6
POP6_SA_1	3846.94	3449.71	3030.76	87.86	76.14	10.59
POP6_SA_11	3114.99	2753.38	2438.34	88.56	74.73	8.68
POP6_SA_4	2756.96	2507.18	2250.94	89.78	73.76	8.12
POP6_SA_6	3254.57	2923.14	2597.63	88.86	75.19	9.19
POP6_SA_9	4388.5	4058.74	3632.29	89.49	76.82	12.58
POP7_NA_1	4177.21	3734.83	3392.33	90.83	76.89	11.74
POP7_NA_1-2	3052.32	2776.55	2532.56	91.21	75.04	8.98
POP7_NA_21	2788.83	2529.09	2288.9	90.5	74.68	8.15
POP7_NA_3	4384.34	3961.22	3498.23	88.31	77.43	12.02
POP7_NA_7	2552.63	2282.16	2024.34	88.7	74.31	7.25
POP7_SA_11	4019.38	3646.37	3208.73	88	76.63	11.14
POP7_SA_32	3078.52	2759.99	2505.35	90.77	74.4	8.96
POP7_SA_34	3225.97	2921.48	2640.04	90.37	74.41	9.44
POP7_SA_36	2883.85	2625.33	2380.67	90.68	74.01	8.56
POP7_SA_9	4019.54	3616.99	3241.78	89.63	76.27	11.3
POP8_NA_11	4334.14	3888.5	3535.35	90.92	77.26	12.17
POP8_NA_2	2892.12	2613.1	2382.05	91.16	75.27	8.42
POP8_NA_3	3576.72	3240.63	2959.22	91.32	76.79	10.25
POP8_NA_4	3943.13	3526.57	3055.5	86.64	76.53	10.62
POP8_NA_7	3620.45	3241	2176.51	67.16	74.68	7.75
POP8_SA_1	3960.58	3531.78	3193.56	90.42	76.48	11.11
POP9_NA_10	4276.54	3860.62	3444.73	89.23	77.17	11.87
POP9_NA_11	3301.3	2956.71	2684.77	90.8	75.79	9.42

Sample	Sequencing data			Statistics of effective data		
	Raw (Mb)	Clean (Mb)	Mapped (Mb)	Mapped (%)	Coverage (%)	Depth (X)
POP9_NA_3	3535.51	3197	2880.04	90.09	76.37	10.03
POP9_NA_6	3310.8	2936.34	2660.15	90.59	75.66	9.35
POP9_NA_9	3085.77	2684.09	2434.74	90.71	74.75	8.66
POP9_SA_1	3245.75	2938.01	2639.22	89.83	75.01	9.36
POP9_SA_2	3461.11	3149.99	2841.05	90.19	75.41	10.02
POP9_SA_3	2410.91	2210.59	1967.84	89.02	73.2	7.15
POP9_SA_3-2	3529.45	3224.89	2832.22	87.82	75.78	9.94
POP9_SA_7	4082.86	3762.02	3331.05	88.54	76.73	11.55
NA_M_8	3368.25	2828.61	2579.40	91.19	75.4	9.1
NA_M_5	3520.77	2976.90	2715.97	91.23	75.73	9.54
NA_M_6	3578.51	3018.14	2753.46	91.23	75.75	9.67
NA_M_11	5747.18	5130.76	4702.46	91.65	77.54	16.13
NA_M_12	4396.44	3659.59	3335.60	91.15	76.7	11.57

DNA sequencing

To corroborate the results based on the nuclear genome, a fragment (~ 650 bp) of mitochondrial gene (*Cytochrome Oxidase* I, COI) was PCR-amplified and sequenced for each of the *P. xylostella* individuals. The resulting fragments were individually aligned with the BOLD system (http://www.boldsystems.org/index.php/IDS_IdentificationRequest) to confirm their identity. Additional confirmation of sample identity was also performed by conducting a COI-based phylogenetic tree using the sequences from Landry & Hebert (2013) and this study. All samples were individually sequenced with the Illumina sequencing system (HiSeq 2000) in BGI, Shenzhen, China to produce paired-end libraries using an Illumina paired-end library kit. Considering the wide distribution of *P. xylostella*, we aimed to sequence a large number of individual genomes across various geographical locations with an average of ~10× coverage for each of the individuals, which is a strategy previously used for other insect species, such as *Apis mellifera* (Wallberg et al., 2014). Two *Plutella australiana* individuals (Landry and Hebert, 2013) were also sequenced with a 10× coverage, and used as outgroups for comparative analysis of genetic differentiation with the *P. xylostella* populations and construction of the phylogenetic tree.

Data filtering, mapping and SNP calling

Before mapping, all reads were processed for quality control and filtered using Seqtk (https://github.com/lh3/seqtk). Stampy v1.0.27 (Lunter & Goodson 2011), with a fast hashing algorithm and a powerful statistical model for mapping highly polymorphic reads, was employed to map the clean reads onto the *P. xylostella* reference genome using default parameters. Subsequently, mapping results were processed by sorting, indel realignment, and duplicate marking, and low quality filtering using functions in Picard v1.8 (http://picard.sourceforge.net) and GATK2 (DePristo et al. 2011). Sequencing coverage and depth were calculated using the 'DepthOfCoverage' module of GATK2. The sequencing and mapping statistics are summarized in Figure 4.2.

SNP calling was then performed using the GATK HaplotypeCaller with parameters --emitRefConfidence GVCF --variant_index_type LINEAR --variant_index_parameter 128,000. Finally, VariantFiltration was used to filter the SNPs from regions with abnormal sequencing

coverage and constructed a core SNP matrix. The filtering settings were as follows: QD < 2.0 || MQ < 40.0 || ReadPosRankSum < -8.0 || FS > 60.0|| HaplotypeScore > 13.0 || MQRankSum < -12.5.

With the aim of inferring the evolutionary origin of *P. xylostella* in the Americas, a sister species, *P. australiana*, was chosen and used as an outgroup species. We identified segregating loci of *P. australiana* (outgroup species) from the *P. xylostella* SNP database, and assembled the consensus sequence for the *P. austrialiana* individuals with the *P. xylostella* genome using SOAPSNP (http://soap.genomics.org.cn/soapsnp.html). The sequencing reads were then aligned to *P. xylostella* genome using Stampy v1.0.27 (Lunter & Goodson 2011) with default parameters. UnifiedGenotyper was used to call genotypes across the two *P. australiana* individuals, and VariantFiltration was used to filter variant calls based on the following parameters: QD < 20.0 || ReadPosRankSum < -8.0 || FS > 10.0 || QUAL < MEANQUAL.

Regional patterns of genetic variation

The statistics of distribution of identified SNPs in different genomic regions are summarized in Table 4.3. Values of nucleotide diversity (Pi, defined in Nei & Li 1979) and numbers of SNPs were calculated for every 50-kb non-overlapping window of genome for both North American and South American collections of *P. xylostella*. A saturation curve of SNPs was developed against genomes of the collected *P. xylostella* individuals. SNP numbers were computed with an increment of 5 individual genomes, and such a procedure was performed with five replicates.

We compared patterns of linkage disequilibrium (LD) and minor allele frequency (MAF) between the collections from North America and from South America. To measure LD levels, the squared correlation (r^2) between any two of the alleles was calculated using PopLDdecay (https://github.com/BGI-shenzhen/PopLDdecay), with parameters "-MaxDist 2 -Miss 0.5 -MAF 0.01". The resulting values of r^2 were then plotted against pairwise SNP distances to show the linkage-disequilibrium patterns across the *P. xylostella* genomes.

Construction of the phylogenetic trees

Phylogenetic relationships of nuclear and mitochondrial SNPs were analyzed for 177 individual

samples of *P. xylostella* with two samples of *P. australiana* used as a outgroup, respectively, using the neighbor-joining (NJ) method, implemented in MEGA 6.06 (Tamura et al., 2013) with 1,000 bootstrap replicates.

Wolbachia plutWB1 was reported to twist maternal genetic structure of *P. xylostella* (Delgado and Cook, 2009). We thus examined all of the mitochondrial SNPs to check for infection by *plutWB1*. Reads from each of the infested individuals were mapped onto the *plutWB1* sequence (Genbank accession number EU833358.1) to confirm their infection status.

Demographic history

The demographic history of all *P. xylostella* individuals from the Americas was predicted using SMC++, a recently developed approach with the highest accuracy to infer demographic variation of a large sample size (Schiffels and Durbin, 2014; Terhorst et al. 2017). A mutation rate (m) of 3×10^{-9} per base pair, from *Drosophila melanogaster* by assuming a generation time of one month was applied in our case (Terhorst et al. 2017).

We also tried to infer the earlier evolutionary history of *P. xylostella* using another approach, pairwise sequential Markovian coalescence (PSMC) based on the distribution of SNPs (Li and Durbin, 2011). The generation time (g) was set as an estimate of 0.083 years. We used a mutation rate (m) of 0.53×10^{-8}, from *Apis mellifera* (Wallberg et al. 2014).

Genomic signatures of local adaptation and natural selection

SNPs from North American and South American collections were used to detect genetic variants involved in local adaptation using an allele-frequency-based approach (Wallberg et al. 2014). F_{ST} estimated at every SNP was calculated for such pairwise comparisons using the method presented in Weir and Cockerham (1984) and VCFtools v0.1.12 (Danecek et al. 2011). We identified genes associated with the differentiated SNPs taken from the top 0.1% of the F_{ST} distribution for each of the pairwise comparisons as candidates for positive selection. Among the group of genes under positive selection, we then selected those with SNPs in coding regions in terms of the list of preferentially expressed genes associated with environmental perception, detoxification of plant secondary metabolites and defense compounds, and insecticide resistance

in the larvae stage (You et al. 2013), and predicted the potential change to protein structure when non-synonymous mutations were identified.

We used MODELLER 9.16 (Sali and Blundell, 1993) to create the homology models of selected DBM proteins. Models of two P450 enzymes CYP12A2 (CCG007339) and CYP9F2 (CCG003485 were built based on human microsomal P450 3A4 (PDB: 1TQN), and UDP-glucuronosyltransferase (UGT) 2B15 (CCG006292) models were built based on *Arabidopsis thaliana* glucosyltransferase (PDB: 2VCH). UCSF Chimera (Pettersen et al. 2004) was used to analyze the interaction networks and prepare the figures.

4.3 Results

Evolutionary and demographic history

Based on the genome-wide analysis of single nucleotide polymorphism (SNP) variation (approximately 21 million SNPS), we demonstrated an evolutionary relationship of *P. xylostella* populations in the Americas. Using *Plutella australiana* as an outgroup, South American populations were found to be the most basal lineage with populations in North America forming independent and derived lineages, based on evidence of both nuclear and mitochondrial SNPs (Figure 4.7; Figure 4.8). Two populations from the northern part of South America (Colombia and Venezuela) were evolutionarily closer to the North America moths by having a more recent common ancestor in terms of the topology of nuclear and mitochondrial phylogeny. Analysis of genetic structure also verified similar genetic components shared between populations from northern South America and North America (Figure 4. 9). The mitochondrial SNPs-based phylogeny presented a basal clade consisting of all *P. xylostella* individuals infected by a specific *Wolbachia* strain, *plutWB1* (Delgado and Cook 2009). In addition, individuals from two South American populations (from Peru and Chile, respectively) are all infected with the *pluWB1*, likely resulting from a *Wolbachia* sweep on mitochondrial genomes in these populations.

Possible origins (source populations) of annual migration of *P. xylostella* in the Americas were predicted by presenting the distribution of two dominating COI-haplotypes. In North America, the dominating haplotype can be found in the populations across the US and Canada (excepting

the southwestern US) while in South America, the dominating haplotype can be detected across the entire continent, excepting the central part (Figure 4.10).

A recently developed sequential-Markov-coalescent-based approach, SMC++ was applied to identify the historical variation of demography for *P. xylostella* in the Americas (Figure 4.11). We found that the *P. xylostella* in both North America and South America underwent a significant population boom over the recent 100 - 400 years, and a relatively slow decline occurred over the most recent 100 years, while the PSMC-based inference of demographic variation (Figure 4.12) suggested an earlier history with *P. xylostella* populations undergoing a significant decline after the last glacial period, approximately 20,000 years ago (Hulton et al. 2002).

Regional patterns of genetic variation

Approximately 21 million SNPs (Table 4.3) were obtained from a genomic dataset with 1,773 coverage generated from 174 *P. xylostella* individuals collected across 38 different geographical locations in 12 countries of the Americas (Table 4.2). Genomic regions with higher numbers of variants are comparable between *P. xylostella* collections from North and South America (Figure 4.3). In contrast to a relatively higher level of variation of the SNP sequences (P_i = 0.0016) among the North American populations, South American populations possessed a lower level of variation (P_i = 0.0010; Table 4.4; Figure 4.3) although they shared a high degree of polymorphic SNPs (~3.85 million SNPs) among the *P. xylostella* populations (Table 4.4). Our analysis based on the SNP saturation curve against *P. xylostella* individuals revealed a semiparabolic pattern as shown by a consistent positive correlation between the number of SNPs and the number of scaling-up individuals (Figure 4.4), suggesting a high-level of genetic variation among the *P. xylostella* populations in the Americas. Continent-based linkage disequilibrium (LD, Figure 4.6) and minor allele frequencies (MAF, Figure 4.5) were presented. A majority of segregation sites were found to be at very low frequency for all populations in the Americas, i.e. SNPs with frequency < 0.2 account for more than 80% in both SA and NA (Figure 4.5). The linkage disequilibrium (LD) measured by r^2 exhibited a similarity between the SA and NA, declining sharply at the first phase of pairwise SNP distance (PD) (PD = 0 ~ 200 bp) and then tending to be stable at other phases (PD > 200 bp). The maxima of r^2 were observed at the very beginning of the first phase (PD = 1 bp), ranging from 0.33 (NA) to 0.49 (SA). Higher value of linkage

disequilibrium (LD) was observed in South America, but an extremely fast decay of LD within a short physical distance was observed for *P. xylostella* from both SA and NA (~50% reduction in the r^2 linkage statistic within only 26-35 bp; Figure 4.6).

Genomic signatures of local adaptation

Aiming to uncover genetic variants responsible for phenotypes associated with local adaptation, populations of *P. xylostella* from NA and SA (individuals from Colombia and Venezuela were excluded) were grouped for pairwise comparisons. Genes overrepresented in the top 0.1% results from the single-SNP-based F_{ST} scan for such a comparison were identified as candidates for positive selection. Highly localized genes (under strong positive selection pressure) associated with environmental perception and insecticide resistance in larvae stage are summarized in Table 4.5. Among the list of loci overrepresented in the top 0.1% results of the comparison between two groups of populations from North American and South American, signatures of strong localized selection can be identified in various parts of the genome, and a typical example is presented in Figure 4.13. A set of four olfaction-related genes, CCG003550.1; CCG003552.1; CCG003553.1; and CCG003554.1, are strongly localized in South American. Ability of olfactory/gustatory reception is therefore likely to differ between moths from North America and South America, resulting from highly differentiated SNPs identified in *P. xylostella* individuals from the different continents.

Examples of genes with significant change to protein structure resulting from strong localized selections are shown in Figure 4.14, 4.15. I predicted the 3D structural models for several DBM proteins that show major sequence variations between the North American and South American populations by the PHYRE2 server (Kelley et al.2015). Three proteins that have reliable homologous models and mutations predicted to be located in buried positions were selected for further investigation. I built the homology models of DBM P450 CYP-12A2 (CCG003485.1), CYP-9F2 (CCG007339.1), and UDP-glucuronosyltransferase (UGT) 2B15 (CCG006292.1) using Modeller 9.16 (Figure 4.15; Sali and Blundell, 1993). Five mutations (K82R, G84S, M85I, F87L and F89Y) in CYP-12A2 cluster in a loop that interacts with the heme molecule in the active site (HEME) (Figure 4.15A, B). They cause a clear conformational change of the loop and directly affect a number of residues surrounding HEME. More importantly, the side chain of

Met85 directly interacts with HEME, and the mutation to isoleucine shortens the side chain and abolishes the interaction. I predict that these mutations very likely modify the function of DMB CYP-12A2 protein. In CYP-9F2, mutations I36V and L48T affect the binding of HEME indirectly through their impacts on residues N364 and R336 respectively; while mutation K25A changes the local conformation of a loop (Figure 4.15C, D). In UGT-2B15, A56S affects the binding of UDP allosterically through residue W253; while E441D changes the conformation of several residues in the local interaction network (Figure 4.15E-G). Although the overall structures of the models with North American and South American sequences are similar, the small conformational changes identified near the critical active sites might be enough to fine tune the activity of these enzymes.

Table 4.3 Distribution of SNPs across different genomic regions

Genomic region	Number	Percentage (%)
Intergenic	9,750,196	46.4117
Exon	2,093,007	9.9629
Intron	6,340,943	30.1834
Start_Codon	1,112	0.0053
Stop_Codon	1,907	0.0091
Splice_Site	3,007	0.0143
Upstream	1,408,291	6.7036
Downstream	1,409,590	6.7098
Total	21,008,053	

Table 4.4 Polymorphism parameters of the *P. xylostella* in South America (SA) and North America (NA)

	Pi	SNP number	Shared SNPs
SA	0.0010	7,581,343	3,849,538
NA	0.0016	17,276,248	3,849,538

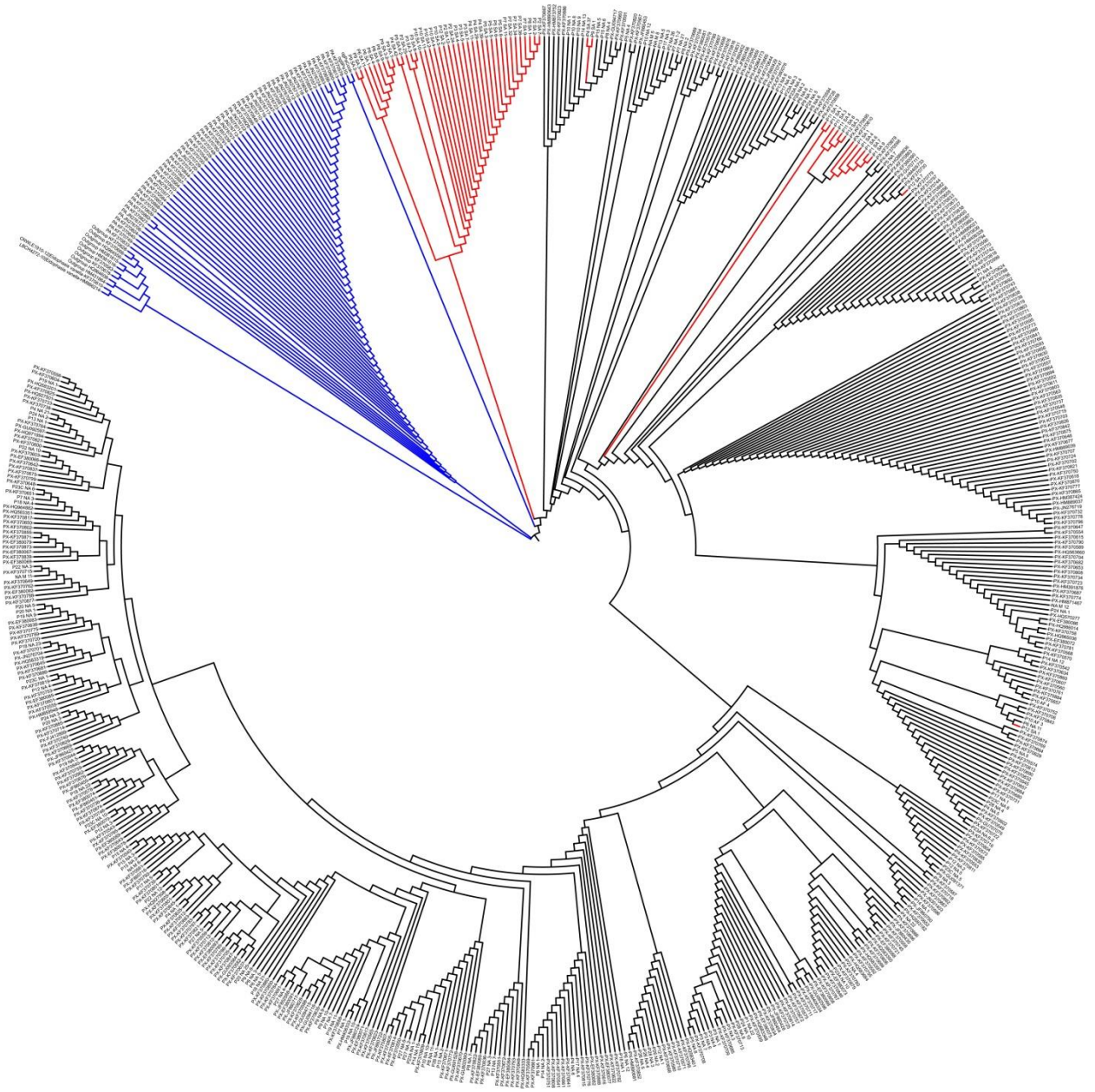


Figure 4.2 Neighbor-joining tree of the COI-gene for all collected specimens in this study and sequence information from Landry and Hebert (2013). The red branches represent individuals from South America, and the blue branches represent individuals of five *Plutella* species (*Plutella armoraciae, Plutella porrectella, Plutella geniatella, Plutella notabilis, Plutella hyperboreella*) and *Eidophasia vanilla*.

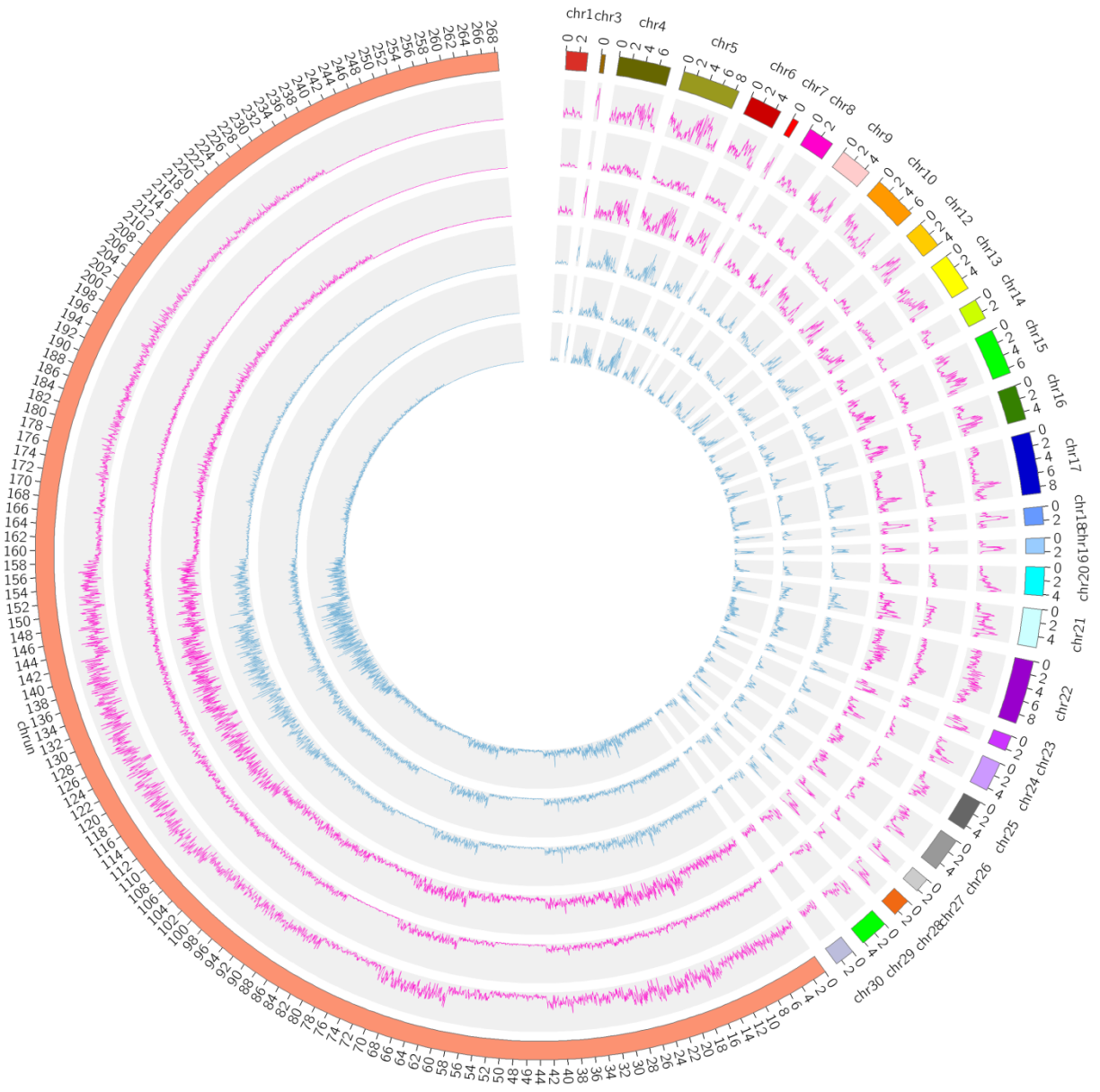
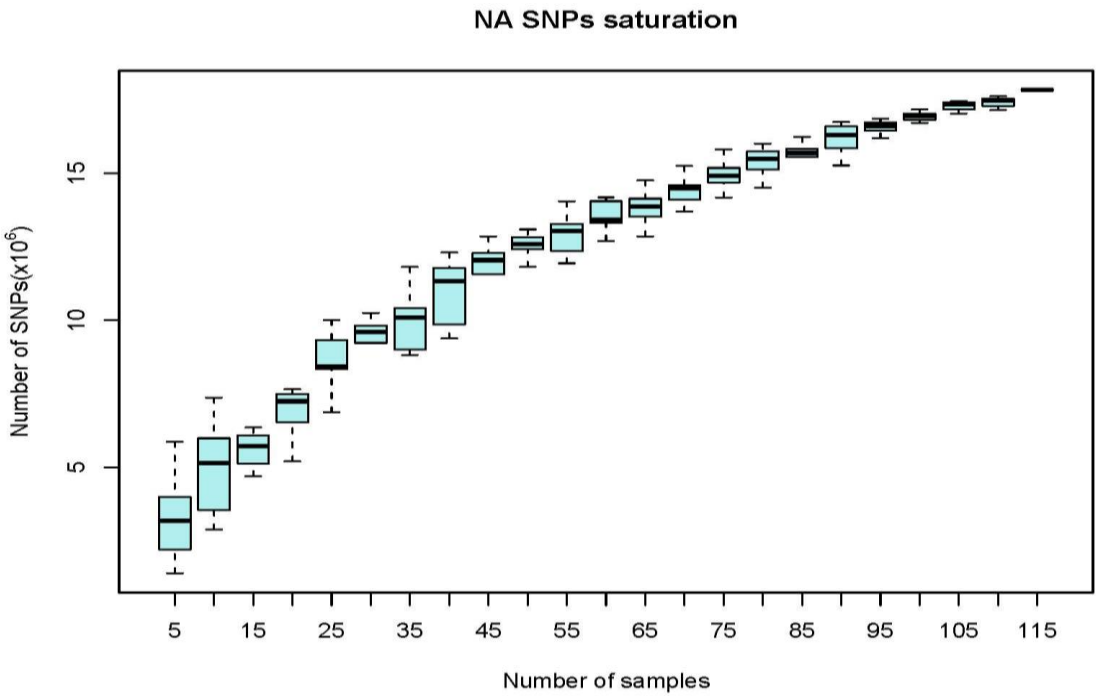


Figure 4.3 Genomic variations of sequenced *P. xylostella* populations. The outermost circle shows the reference genome assembly with a 1Mb unit scale. Scaffolds that could be assigned to linkage groups are joined in arbitrary order to generate the partial sequences of 28 chromosomes, and the orange segment represents the scaffolds that were unable to be assigned (Chrun, You et al. 2013). Number of SNPs and Nucleotide diversity (Pi) are shown in pink and blue, respectively. Three outermost tracks (shown in pink) depict the ratio of SNPs in every 50-kb window in the Americas, South America, and North America, respectively; while the three innermost tracks (shown in blue) depict the nucleotide diversity (Pi) in the Americas, South America, and North America, respectively.

A



B

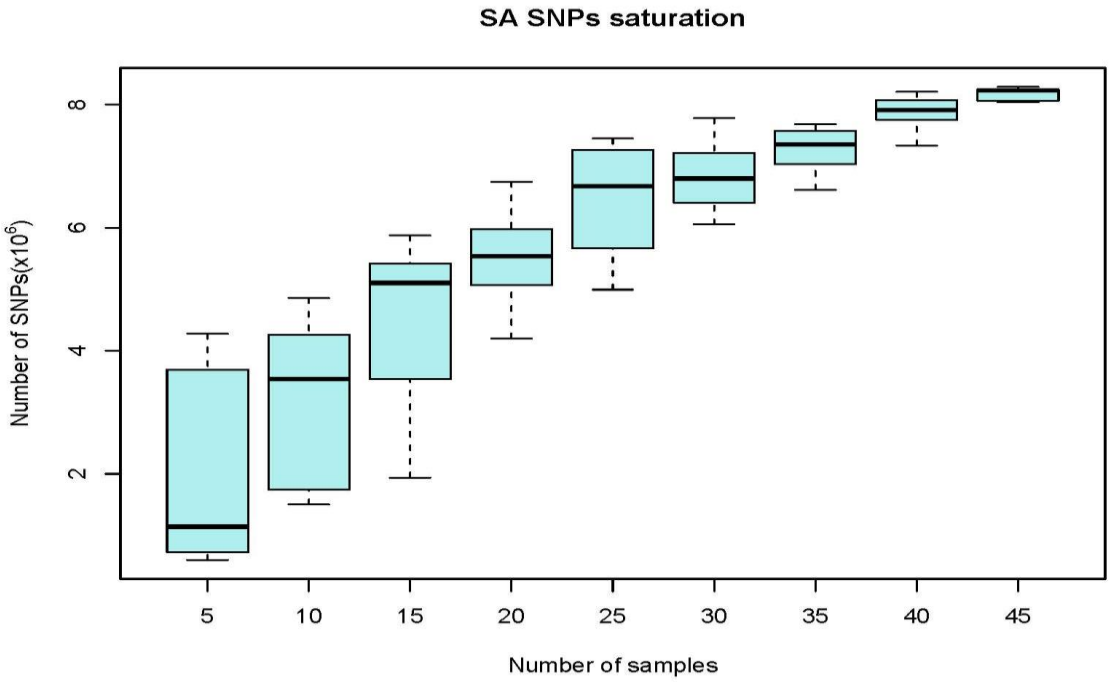


Figure 4.4 SNP saturation curve based on independent samplings from sampled *P. xylostella* individuals collected in North America (A) and South America (B). Each of the samplings was performed with five replicates and the relevant numbers of SNP computed, scaling up by an increment of five individuals. The histogram shows the SNP variation and deviation of five replicates for each sampling.

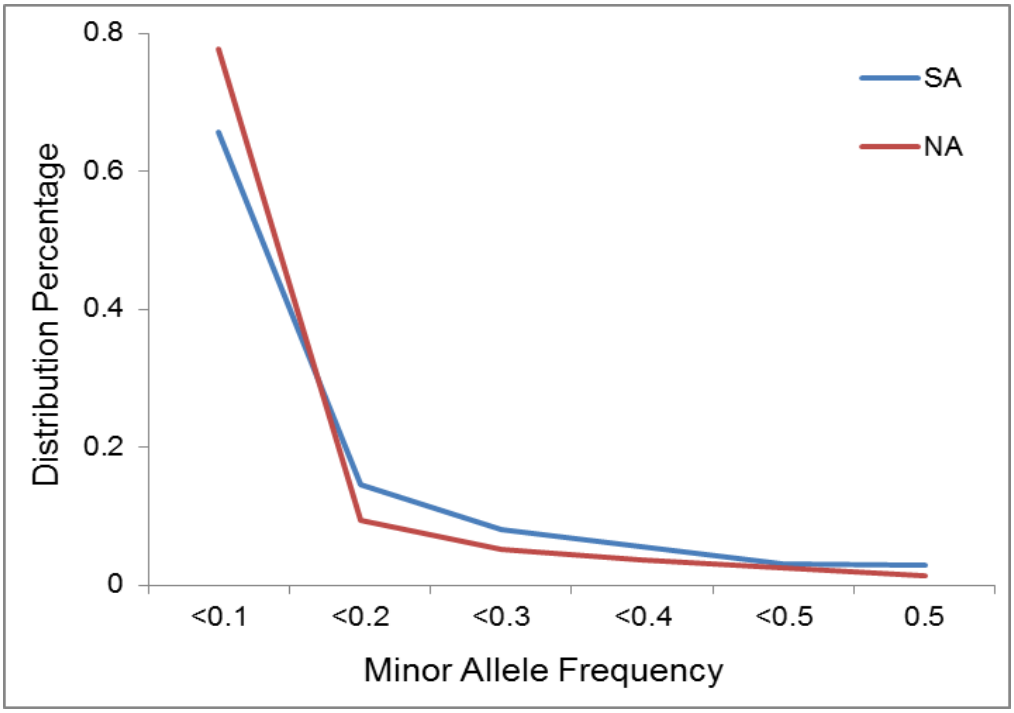


Figure 4.5 Genome-wide distribution of the minor allele frequency in the NA and SA colonies of *P. xylostella*.

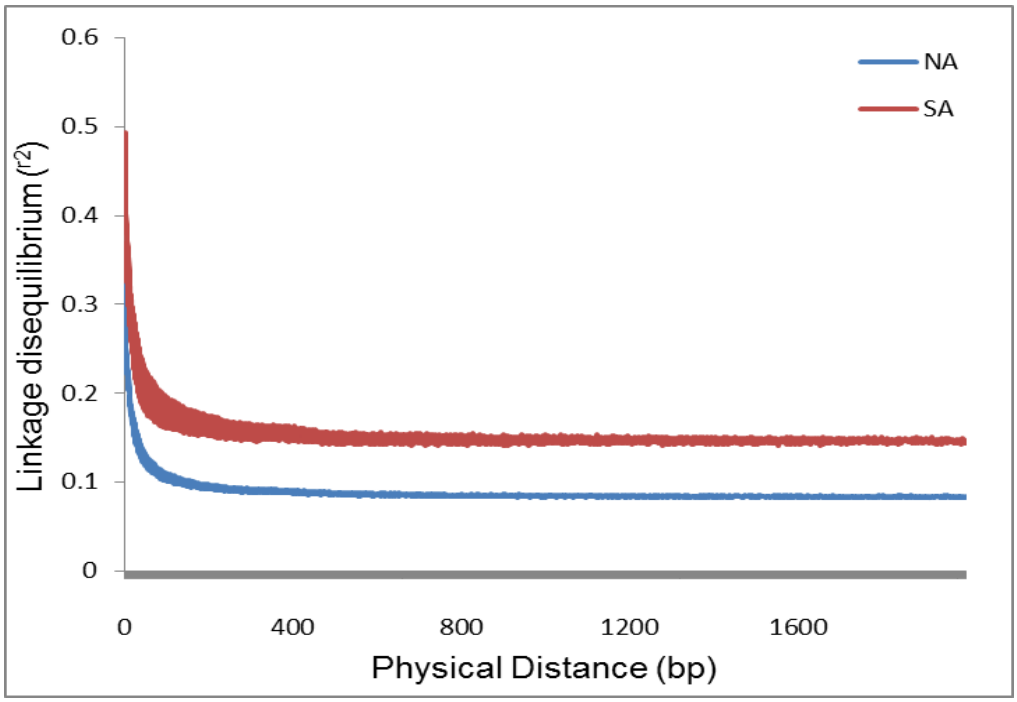


Figure 4.6 Linkage-disequilibrium patterns against physical distance (bp) based on the *P. xylostella* genome-wide SNPs from NA and SA.

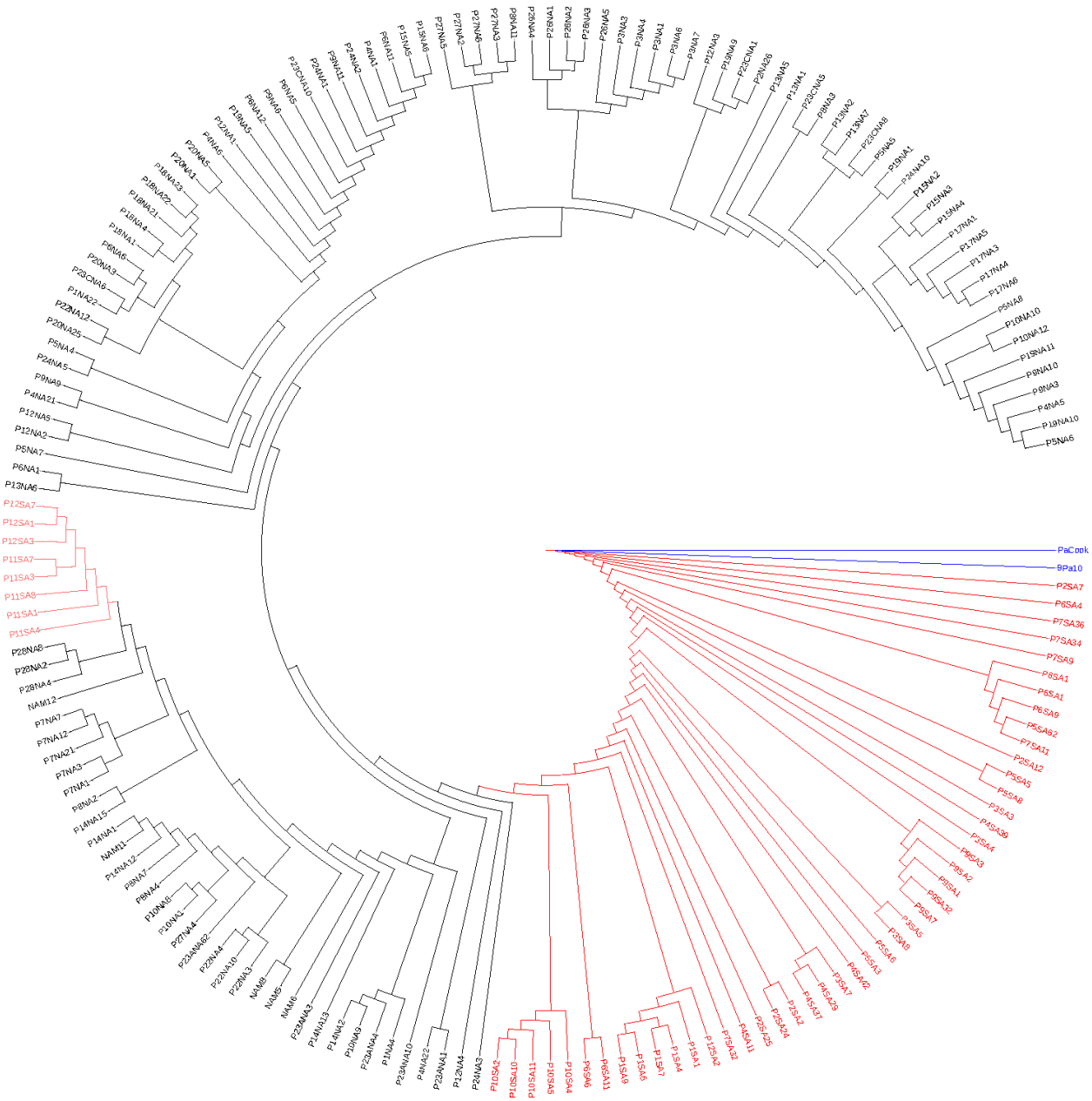


Figure 4.7 The phylogenetic tree constructed using neighbor-joining algorithm based on the genome-wide SNPs of *P. xylostella*. The red branches represent individuals from South America, and the black branches represent individuals from North America. Two *P. australiana* individuals were used as outgroups, and colored in blue.

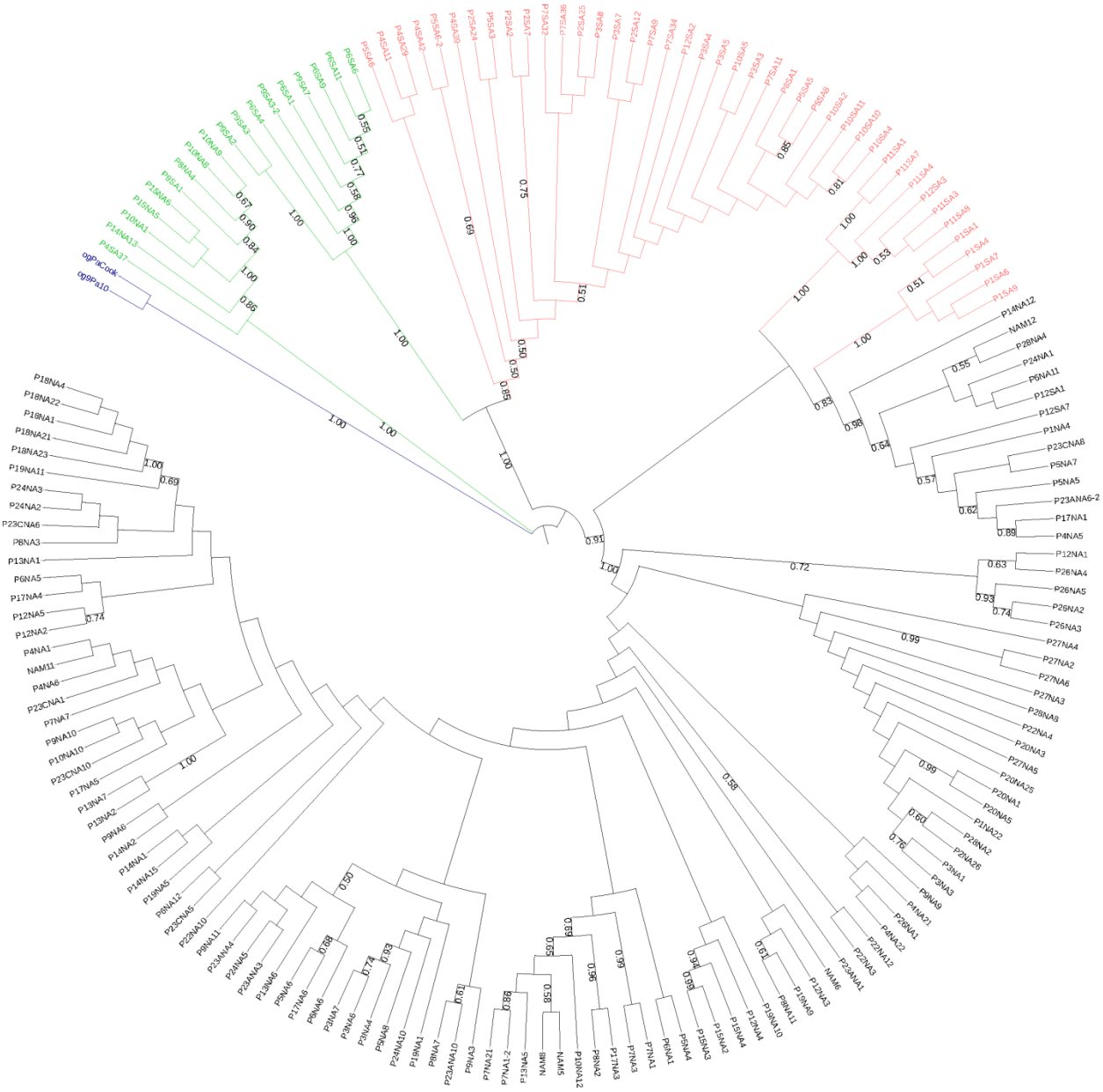


Figure 4.8 The phylogenetic tree constructed using NJ algorithm based on mitochondrial genome-wide SNPs of *P. xylostella*. The green branches represent individuals infected by *Wolbachia plutWB1*, the red branches represent individuals uninfected by *Wolbachia plutWB1* from South America, and the black branches represent individuals uninfected by *Wolbachia plutWB1* from North America. Two *P. australiana* individuals were used as outgroups, and colored in blue.

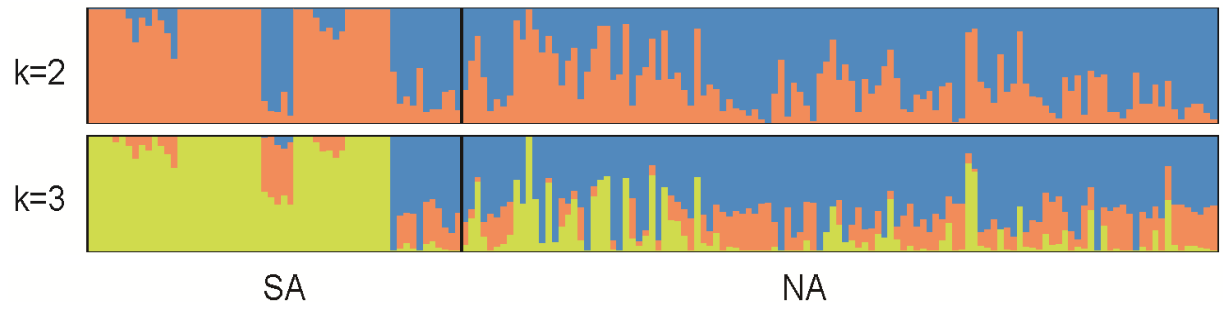


Figure 4.9 Genetic structure of *P. xylostella* populations from North America and South America. Colors in each column represent ancestry proportion over range of population sizes (K=2/K=3).

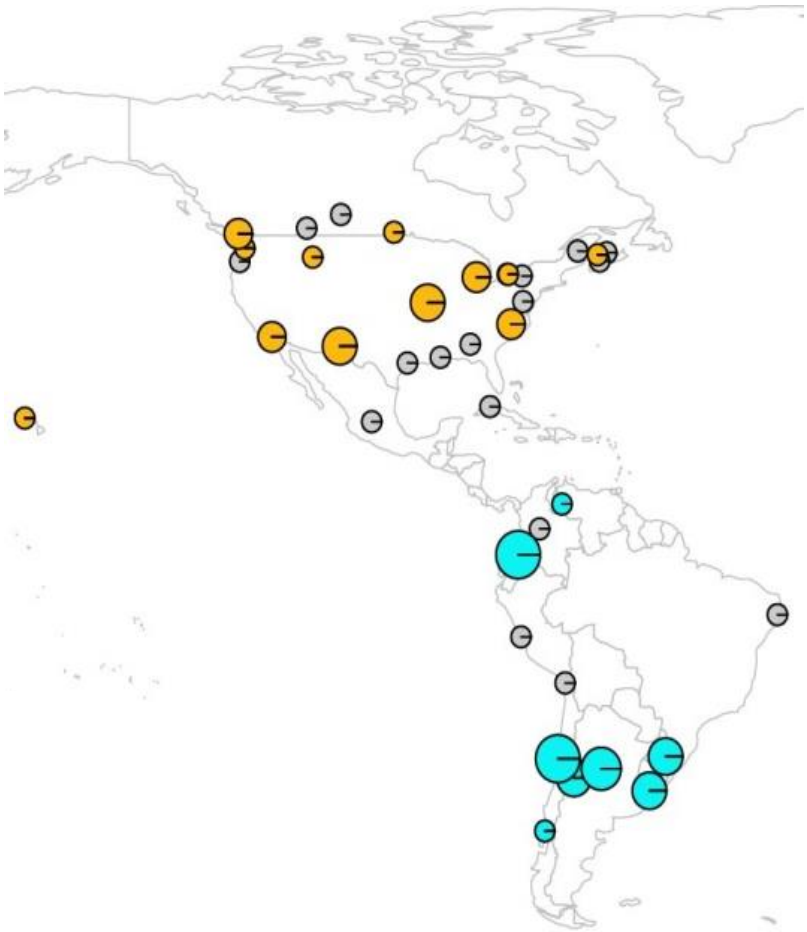


Figure 4.10 Distributions of two dominant haplotypes (represented as yellow and blue-green, respectively) of mitochondrial gene COI. Grey represents the composition of other haplotypes.

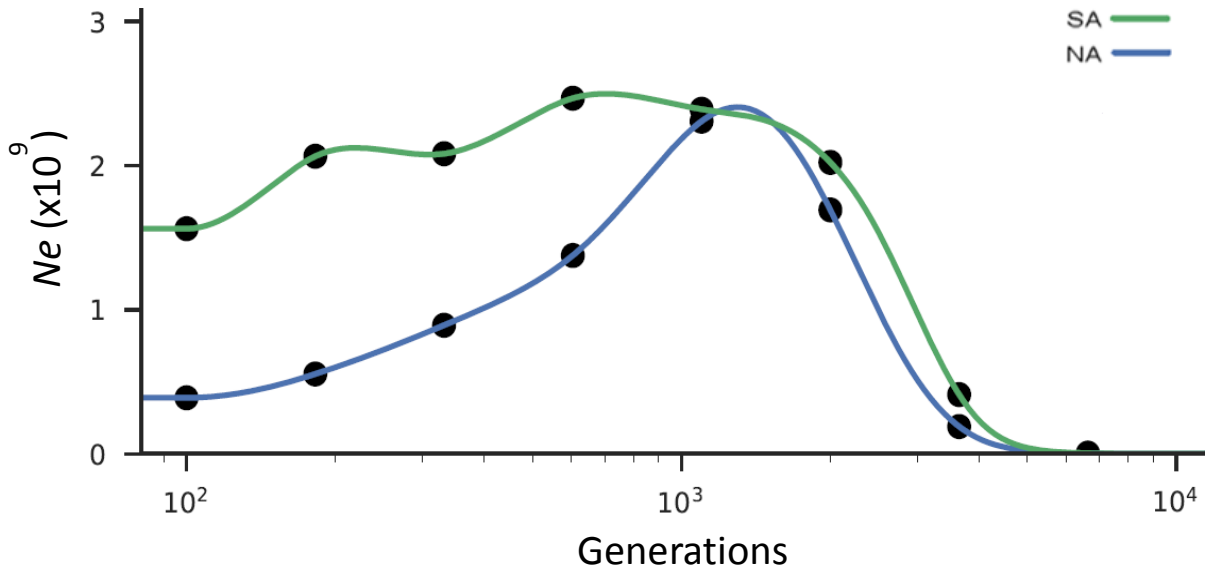


Figure 4.11 Demographic history of the *P. xylostella* colonies in the Americas inferred by SMC++.

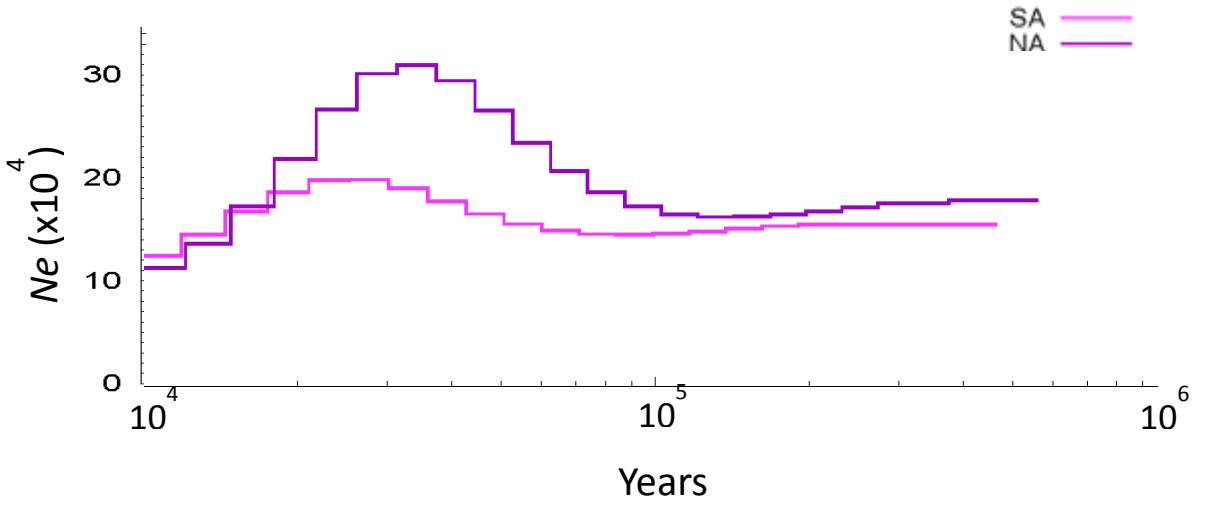


Figure 4.12 Demographic history of the *P. xylostella* in the Americas predicted with a pairwise sequentially Markovian coalescent (PSMC) model.

Table 4.5 InterPro-based annotations on preferentially expressed genes in larvae with highly differentiated SNPs in coding regions

Gene	Position	Start	End	InterPro
CCG000163.1	scaffold_10	1427959	1440157	IPR001140; ABC transporter, transmembrane domain IPR003439; ABC transporter-like IPR003593; ATPase, AAA+ type, core IPR011527; ABC transporter, transmembrane domain, type 1 IPR017871; ABC transporter, conserved site IPR017940; ABC transporter, integral membrane type 1
CCG000515.1	scaffold_104	661285	663365	IPR002018; Carboxylesterase, type B IPR019826; Carboxylesterase type B, active site
CCG001209.1	scaffold_114	851913	853847	IPR010582; Catalase-related immune responsive IPR011614; Catalase, N-terminal IPR018028; Catalase-related subgroup IPR020835; Catalase-like domain, haem-dependent
CCG001815.1	scaffold_127	10199	20819	N/A
CCG002290.8	scaffold_134	680313	694006	IPR000794; Beta-ketoacyl synthase IPR001031; Thioesterase IPR001227; Acyl transferase domain IPR009081; Acyl carrier protein-like IPR011032; GroES-like IPR013149; Alcohol dehydrogenase, C-terminal IPR014030; Beta-ketoacyl synthase, N-terminal IPR014031; Beta-ketoacyl synthase, C-terminal IPR014043; Acyl transferase IPR016035; Acyl transferase/acyl hydrolase/lysophospholipase IPR016036; Malonyl-CoA ACP transacylase, ACP-binding IPR016038; Thiolase-like, subgroup IPR016039; Thiolase-like IPR016040; NAD(P)-binding domain IPR018201; Beta-ketoacyl synthase, active site IPR020801; Polyketide synthase, acyl transferase domain IPR020841; Polyketide synthase, beta-ketoacyl synthase domain IPR020843; Polyketide synthase, enoylreductase
CCG002416.1	scaffold_137	513599	532977	IPR001140; ABC transporter, transmembrane domain IPR003439; ABC transporter-like IPR003593; ATPase, AAA+ type, core IPR011527; ABC transporter, transmembrane domain, type 1 IPR017871; ABC transporter, conserved site IPR017940; ABC transporter, integral membrane type 1
CCG002723.1	scaffold_140	953589	955357	IPR001128; Cytochrome P450 IPR002401; Cytochrome P450, E-class, group I IPR017972; Cytochrome P450, conserved site
CCG003246.1	scaffold_15	2271373	2275194	IPR001128; Cytochrome P450 IPR002401; Cytochrome P450, E-class, group I IPR017972; Cytochrome P450, conserved site

Gene	Position	Start	End	InterPro
CCG003448.7	scaffold_153	273339	287095	IPR002018; Carboxylesterase, type B IPR019826; Carboxylesterase type B, active site
CCG003485.1	scaffold_154	219752	226769	IPR001128; Cytochrome P450 IPR002403; Cytochrome P450, E-class, group IV IPR017972; Cytochrome P450, conserved site
CCG003554.1	scaffold_156	494198	495170	IPR004117; Olfactory receptor, Drosophila
CCG003553.1	scaffold_156	484924	488237	IPR004117; Olfactory receptor, Drosophila
CCG003550.1	scaffold_156	443625	454909	IPR004117; Olfactory receptor, Drosophila
CCG003649.1	scaffold_159	582419	585425	IPR002018; Carboxylesterase, type B IPR019826; Carboxylesterase type B, active site
CCG004174.1	scaffold_168	134947	152127	IPR001394; Peptidase C19, ubiquitin carboxyl-terminal hydrolase 2 IPR018200; Peptidase C19, ubiquitin carboxyl-terminal hydrolase 2, conserved site
CCG004815.1	scaffold_183	518602	520672	IPR002018; Carboxylesterase, type B
CCG005591.1	scaffold_200	68221	86053	IPR001140; ABC transporter, transmembrane domain IPR003439; ABC transporter-like IPR003593; ATPase, AAA+ type, core IPR011527; ABC transporter, transmembrane domain, type 1 IPR017871; ABC transporter, conserved site IPR017940; ABC transporter, integral membrane type 1
CCG005628.1	scaffold_201	357248	358645	IPR010582; Catalase-related immune responsive IPR011614; Catalase, N-terminal IPR018028; Catalase-related subgroup IPR020835; Catalase-like domain, haem-dependent
CCG005902.4	scaffold_21	1287549	1289300	IPR001128; Cytochrome P450 IPR002401; Cytochrome P450, E-class, group I IPR017972; Cytochrome P450, conserved site
CCG005900.1	scaffold_21	1278550	1279356	IPR001128; Cytochrome P450 IPR002402; Cytochrome P450, E-class, group II
CCG006286.1	scaffold_221	146916	149236	IPR004045; Glutathione S-transferase, N-terminal IPR004046; Glutathione S-transferase, C-terminal IPR010987; Glutathione S-transferase, C-terminal-like IPR012335; Thioredoxin fold IPR012336; Thioredoxin-like fold IPR017933; Glutathione S-transferase/chloride channel, C-terminal
CCG006292.1	scaffold_221	355785	357943	IPR002213; UDP-glucuronosyl/UDP-glucosyltransferase
CCG006353.1	scaffold_224	37446	46250	IPR004117; Olfactory receptor, Drosophila
CCG006430.1	scaffold_228	56163	61177	IPR002018; Carboxylesterase, type B IPR019826; Carboxylesterase type B, active site

Gene	Position	Start	End	InterPro
CCG006458.1	scaffold_229	298102	301803	IPR004117; Olfactory receptor, Drosophila
CCG007339.2	scaffold_26	566773	570392	IPR001128; Cytochrome P450 IPR002401; Cytochrome P450, E-class, group I IPR017972; Cytochrome P450, conserved site
CCG007344.4	scaffold_26	724089	727173	IPR001128; Cytochrome P450 IPR002401; Cytochrome P450, E-class, group I IPR017972; Cytochrome P450, conserved site
CCG008913.1	scaffold_311	630521	644584	IPR000997; Cholinesterase IPR002018; Carboxylesterase, type B IPR010562; Haemolymph juvenile hormone binding IPR019826; Carboxylesterase type B, active site
CCG009834.1	scaffold_352	169022	191602	IPR001140; ABC transporter, transmembrane domain IPR003439; ABC transporter-like IPR003593; ATPase, AAA+ type, core IPR011527; ABC transporter, transmembrane domain, type 1 IPR017940; ABC transporter, integral membrane type 1
CCG010794.1	scaffold_4	722851	741252	IPR003439; ABC transporter-like
CCG010901.1	scaffold_402	246652	281077	IPR004117; Olfactory receptor, Drosophila
CCG010903.1	scaffold_402	289813	312183	IPR004117; Olfactory receptor, Drosophila
CCG011738.1	scaffold_45	574857	576143	IPR013604; 7TM chemoreceptor
CCG011757.1	scaffold_45	1201538	1203816	IPR002018; Carboxylesterase, type B IPR019826; Carboxylesterase type B, active site
CCG011756.1	scaffold_45	1189765	1201075	IPR002018; Carboxylesterase, type B IPR019826; Carboxylesterase type B, active site
CCG012592.1	scaffold_5	422966	425822	IPR002018; Carboxylesterase, type B IPR019826; Carboxylesterase type B, active site
CCG013728.1	scaffold_58	462696	478688	IPR001140; ABC transporter, transmembrane domain IPR003439; ABC transporter-like IPR003593; ATPase, AAA+ type, core IPR011527; ABC transporter, transmembrane domain, type 1 IPR017871; ABC transporter, conserved site IPR017940; ABC transporter, integral membrane type 1

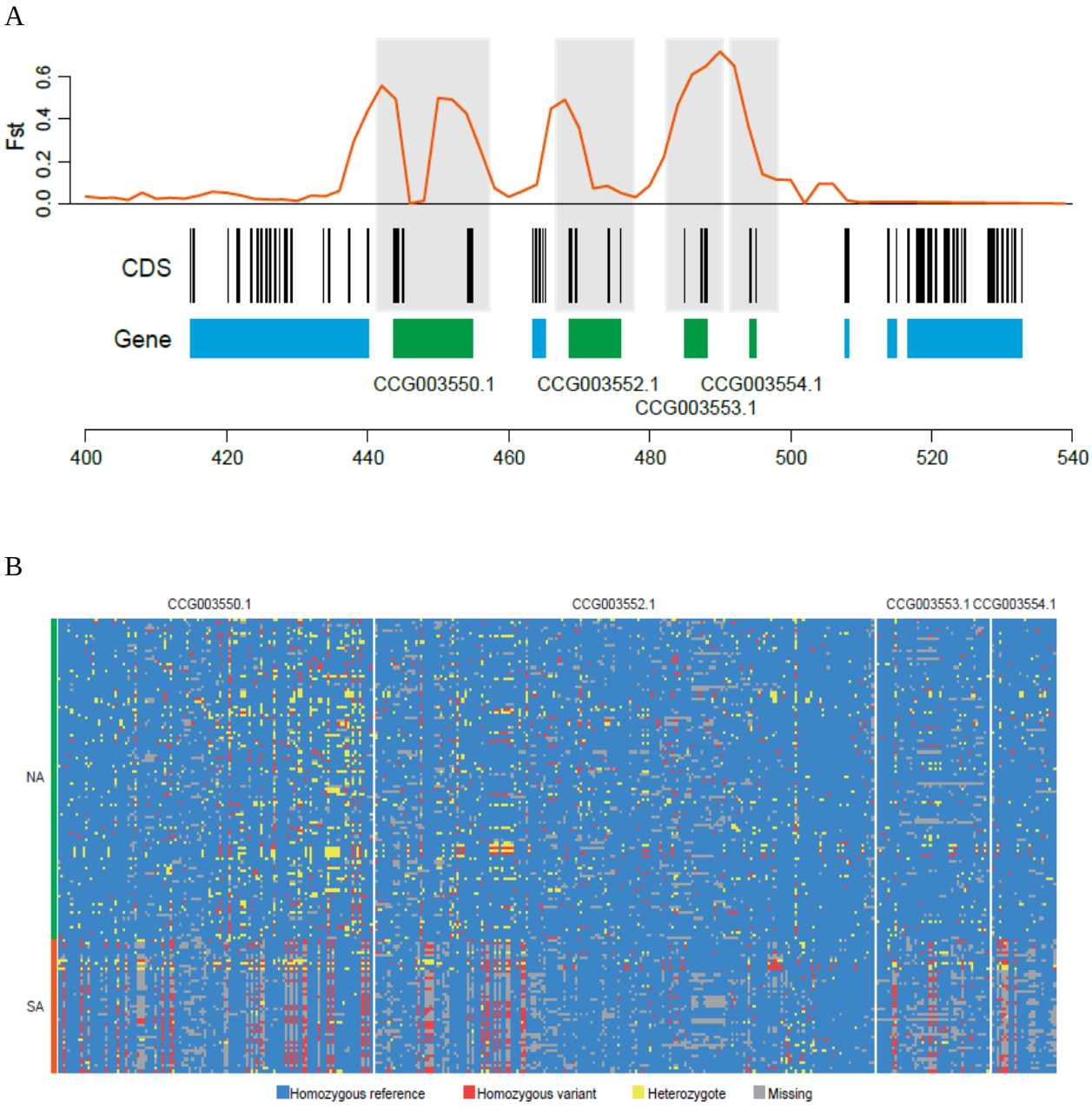


Figure 4.13 Signals of local adaptation associated with olfactory reception. A) F_{ST} value were plotted across genes, CCG003550.1, CCG003552.1, CCG003553.1, and CCG003554.1, and B) Gene models and SNP allele for genes, CCG003550.1, CCG003552.1, CCG003553.1, and CCG003554.1: blue represents homozygous for the reference allele, red represents homozygous for alternative allele, yellow represents heterozygous, and grey represents missing site.

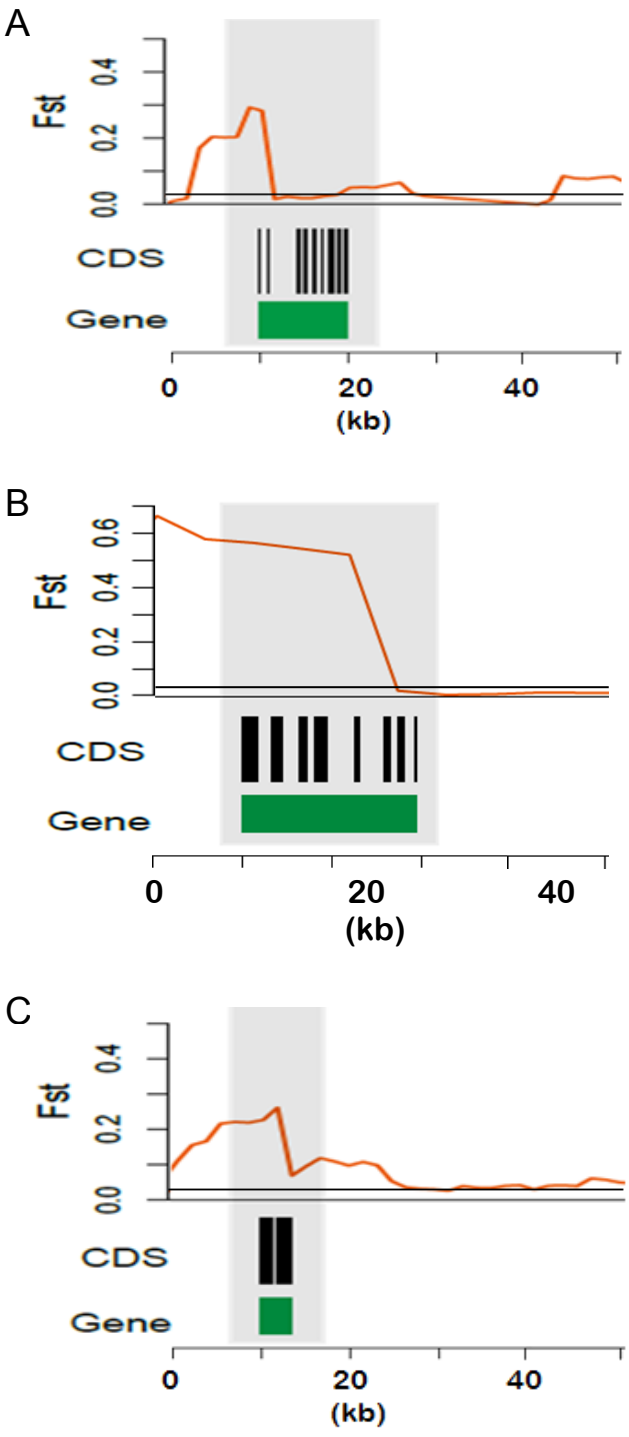


Figure 4.14 F_{ST} statistics presented in a 40kb window between North American populations and South America populations for three selected genes (A: CCG003485.1; B: CCG007339.1, and C: CCG006292.1) with nonsynonymous mutations that cause significant change to protein structure. The black horizontal line represent average F_{ST} value across the entire genome (F_{ST} = 0.0232).

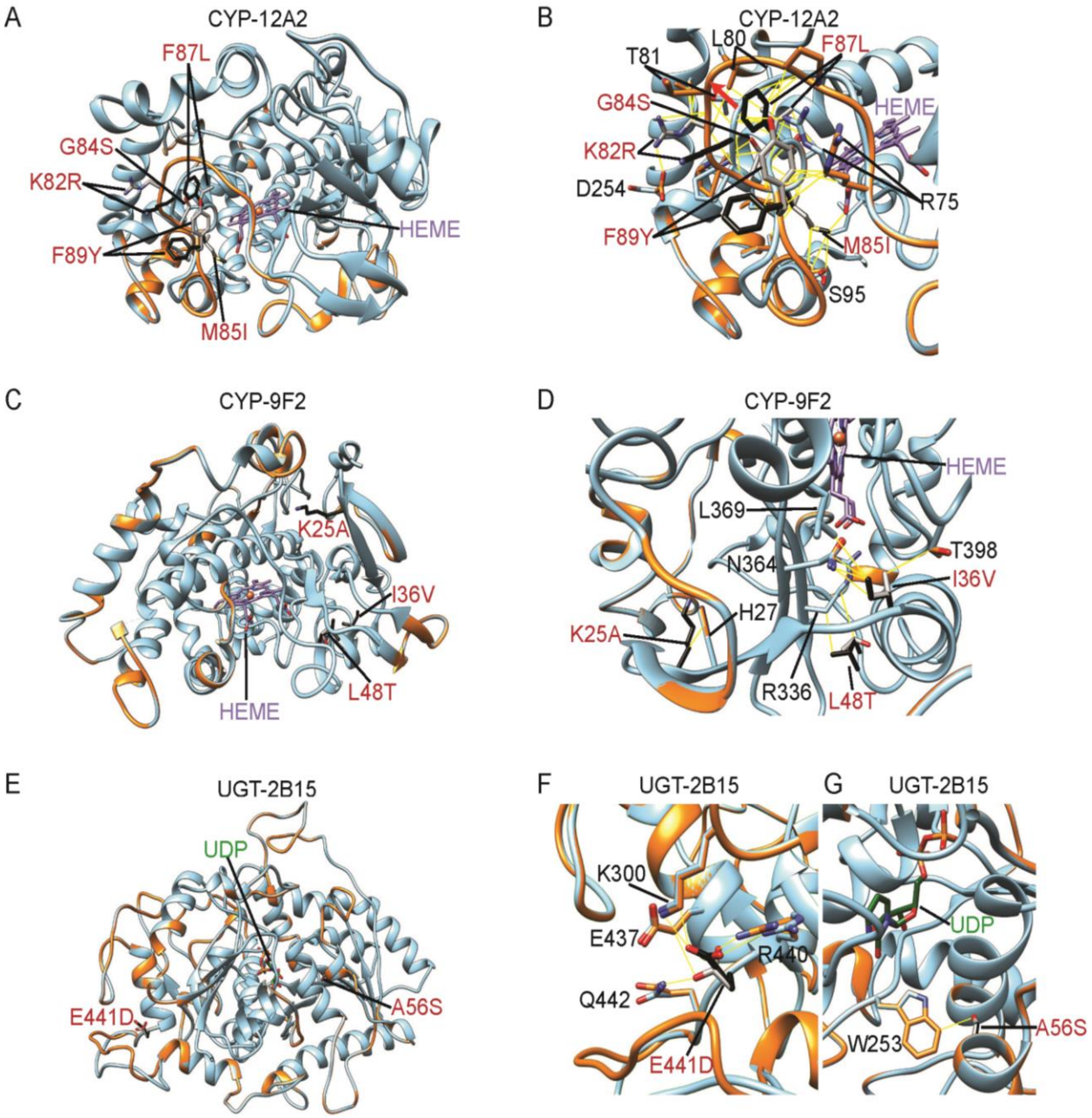


Figure 4.15 Homology models of DBM P450 enzymes CYP12A2 (CCG003485.1), CYP9F2 (CCG007339.1), and UDP-glucuronosyltransferase (UGT) 2B15 (CCG006292.1). The models with North American DBM sequences are colored in blue and the ones with South American DBM sequences are colored in orange. The side chains of the mutated residues are colored in black (North American) and gray (South American). Panels A, C, E show the overall predicted structures; panels B, D, F, and G show the enlarged view of the mutation sites and the residue interaction networks. HEME and UDP molecules from enzyme active sites are shown in purple and green respectively. The contacts between the mutations and the surrounding residues are indicated by yellow lines.

4.4 Discussion

All of our specimens from both North and South America can be confirmed as *P. xylostella*, in terms of the topology of the three phylogenetic trees (Figure 4.2; 4.7; 4.8) by presenting monophyletic groups of all collected specimens with other confirmed *P. xylostella* sequences from previous studies (Landry and Hebert, 2013). Samples from different continents were clustered into different clades, providing evidence of the distinct evolutionary relationships of *P. xylostella* populations in the Americas. My genome-wide analysis suggests that the current broad distribution of the diamondback moth originated in southern parts of South America, followed by dispersal events with a general direction towards the north. *P. xylostella* populations expanded from southern South America into northern South America first and then to North America. Central America (including the Caribbean region) is likely to play a role of significance as a "transit" during *P. xylostella*'s northward expansion. Mexico and coastal areas in the US were the first locations of *P. xylostella* arrival in North America. Such a dispersal scenario was further verified by the evidence that structure of the *P. xylostella* populations from northern South America was genetically comparable to populations from North America. In addition, my finding of the ancestral origin of DBM is also supported by the previous observations as reflected by rich *Plutella* species (Meyrick, 1931), diverse fauna of the *P. xylostella* parasitoids (Furlong et al. 2013), and abundant indigenous *Brassica* host species (Al-Shehbaz, 2010; Al-Shehbaz et al, 2013; Goodson et al 2011; O'Kane & Al-Shehbaz, 2004; Toro-Núñez et al, 2013) in South America.

Some previous studies argue that *P. xylostella* hibernates in plant debris through the winter in temperate regions where *Brassica* hosts are not cultivated year-round (Marsh 1919; Theobald 1926). However, in none of these studies were insects collected during the coldest seasons and sampled out of hibernation (Talekar and Shelton, 1993). Recent investigations tend to emphasize the inability of *P. xylostella* to overwinter in temperate regions (Saito et al.1998; Zalucki and Furlong, 2011), where infestations therefore result from immigration. In this study, no significant geographical differentiation was observed within the North American or South American populations, except for two populations from northern South America. The wide and relatively even distribution of dominant haplotypes across the two continents in the Americas provides

further evidence for migration, as demonstrated in other migratory species (Dallimer et al. 2003; Llewellyn et al. 2003; Uthicke, 2003; Endersby et al. 2006; Lyons et al. 2012)

Outbreaks resulting from northward advections have been reported in North America for insect species, including *P. xylostella* (Rogers *et al*. 1986; Putnam and Burgess, 1977; Smith and Sears, 1982). Additionally, observation and speculation of annual long-distance migration of *P. xylostella* have been documented across Europe, North America, East Asia and Oceania (Chapman et al. 2002; Philip and Mengersen, 1989; Endersby et al. 2006; Honda, 1992; Wei et al. 2013). The northward recolonization is the most common phenomenon across North America and East Asia, especially moving from year-round persistence areas into areas that are only seasonally suitable for growth and development (Dosdall et al. 2004; Furlong et al. 2013, Wei et al. 2013). However, no consensus has been reached about the site(s) of origin of *P. xylostella* populations invading the northern US and Canada. My COI-haplotype-based analysis revealed potential source populations (i.e. from Southwest US) for the annual dispersal for *P. xylostella* in North America, by showing genetic connections of populations with the same haplotype component (Figure 4.10), and *Brassica*-based landscapes in the central US are believed to facilitate such a long-distance movement by providing the necessary food and habitats.

This is the first study to explore *P. xylostella*'s annual migration in South America. The detailed migration route of *P. xylostella* might be complicated but with general north-south directions, considering patterns of prevailing winds (Grimm et al. 2005; Dias and Carvalho, 2017). Movement of *P. xylostella* across the southern parts of South America is in favor of intermixing of the *P. xylostella* populations within the sites of evolutionary origin.

The enormous change of environment during the last glacial period, approximately 20,000 years ago, likely contributed to a significant decline of *P. xylostella* populations during that time, as identified in this study (Hulton et al. 2002). Such an estimated population decline is consistent with the commencement of deglaciation in both the Northern and Southern Hemispheres (Clark et al. 2009). Recent expansion of *P. xylostella* populations in the Americas occurred during the past few hundreds of years, consistent with previous records and estimates of the worldwide distribution of *P. xylostella* (Capinera 2000). The significant expansion of *P. xylostella*

populations, presumably driven by the expansion of ocean freight shipping and international trade in agricultural commodities and products (including seeds and plants of Brassicaceae and unintended *P. xylostella* individuals) (García-Herrera et al. 2005), was further facilitated by the gradual intensification of agriculture and land use over the past century. However, frequent routine application of broad-spectrum chemical insecticides to control diamondback moth infestations may have contributed to the recent depopulation estimated in this study.

My study revealed high diversity of nucleotide variants and mitochondrial haplotypes (COI-based), but with relatively low genome-wide nucleotide diversity (Pi, Π) of *P. xylostella* in both continents (NA and SA). A comparable situation has been reported for other migratory species (Uthicke, 2003; Kraus et al. 2011). In general, more genomic variants were identified in the North American populations of *P. xylostella*. For insect species, it's not uncommon that derived populations are more genetically variable than "source" populations (Wallberg et al. 2014). Rapid decline of LD within short physical distances implies a high recombination rate in *P. xylostella*, and is in concordance with other studies of insect species, especially Lepidoptera (Xia et al., 2009; Wallberg et al. 2014, Zhan et al., 2014).

A plausible explanation for the low nucleotide diversity and high proportion of low-frequency alleles is the relatively recent invasion and subsequent expansion of *P. xylostella* identified in previous studies (Capinera 2000; Wei et al. 2013) and in my analysis, while based on my analyisis, high levels of genetic variation suggested potential for rapid adaptation to various environmental conditions. For example, *P. xylostella* has developed varying degrees of resistance to almost all applied insecticides, and such variation can be observed between geographically-adjacent populations (Caprio and Tabashnik, 1992; Furlong et al.2013). Genetic differences have been identified between pesticide-resistant and susceptible strains (Heckel et al. 1995; Herrero et al. 2001; Zhou et al. 2010), as well as between populations from different temperatures and altitudes (Noran and Tang, 1996). High levels of selection pressure from insecticides and native environments may have become key factors determining the strong signals of localized variation.

Highly localized genes with SNPs in coding regions can be generally categorized into two groups: i) genes associated with insecticide resistance, including ATP-binding cassette (ABC)

transporter, cytochrome P450, glutathione S-transferases (GSTs) and esterase, especially carboxylesterases (COEs); and ii) genes that are potentially involved in DBM-plant interactions, such as olfactory receptors. Gene families of ABC transporters, cytochrome P450s, GSTs, and COEs have been reported to be the four major families having important roles in agrochemical detoxification in insects (Li et al. 2007; Labbé et al. 2011). These four gene families are known to have expanded in the insecticide-resistant strains of *P. xylostella*, compared to those species with little exposure to insecticides, such as *Bombyx mori* (Dermauw et al. 2013; You et al. 2013). The significant differentiation of these genes between the *P. xylostella* from North America and South America might thus imply varying levels of resistance, likely resulting from different regimes of insecticide application. The strong signatures of localized selection identified are likely owing to recurrent and heavy use of a given class of agrochemicals with a similar insecticidal mode-of-action. Selection by exposure to routine use of insecticides might be accelerated by the insect's high fecundity, especially in populations from tropical and subtropical regions (in this case, South America). Furthermore, volatile compounds are known to play important roles in influencing herbivore-plant interactions, and even tri-trophic interactions (Furlong et al. 2013). A series of highly diverged genes involved in olfactory reception identified in *P. xylostella* populations from different continents might indicate a dissimilarity of preference or sensitivity to various profiles of volatile compounds from indigenous crop or noncrop hosts, so that differentiated behavioral responses (e.g. feeding and oviposition) might be observed in moth populations from different geographic areas.

In addition, infection and impacts of *Wolbachia* on *P. xylostella* have not been well documented and studied. Individuals infected by *Wolbachia pluWB1* are all well clustered regardless of their locations, especially by forming a basal clade. *P. xylostella* was predicted to diverge from two other lepidopterans, *B. mori* and *Danaus plexippus* ~124 million years ago, and evolved as a crucifer specialist ~54–90 million years ago (You et al. 2013). I speculate that there is a long history of co-evolution between *P. xylostella* and *plutWB1* in South America, i.e. *pluyWB1* has identified *P. xylostella* with a specific makeup of mitochondrial genome as a host for a long time, and the dispersal of *pluWB1* was facilitated along with the expansion of the *P. xylostella* populations.

4.5 Conclusion

Extensive genome sequencing allows us to characterize the diamondback moth's evolutionary origin, patterns of historical dispersal and annual migration, as well as genome-wide signatures of localized selection to insecticides. Findings in this study confirm the genomic polymorphism and genetic plasticity of *P. xylostella* that provides great capacity for adaptation to different habitats, host plants, and rapid development of resistance to various classes of insecticide (Talekar and Shelton, 1993; Furlong et al., 2013; You et al., 2013), and further enrich our knowledge of ancestral demography and underlying mechanisms that support extremely rapid evolution of environmental adaptation and agrochemical resistance of such a global pest, one of the top species with notorious resistance to pesticides. I not only highlight the South American origin of the diamondback moth, but also the potential contribution of recent population expansion and recurrent annual migration to contemporary genetic configuration and pest status. My inference of strong selection on a number of genes, with potential roles in developing resistance to agrochemicals elucidates underlying mechanisms associated with rapid adaptation of the diamondback moth to intensive insecticide application.

Understanding the dynamics of an insect pest outbreak is important from a management perspective. However, knowing the source and dispersal patterns of a pest is even more crucial to help delineate potential boundaries for control and provide the basis for developing strategies to prevent future expansion, mainly of insecticide resistant strains. In the case of diamondback moth, it is imperative to have a comprehensive knowledge of its genetic structures, and expansion pattern across continents to efficiently define regional control strategies that will be effective in the long term. This is of even greater importance with climate change and global warming, as overwintering of this species could become more frequent in temperate areas, especially in more northerly parts of North America, bringing potentially increased damage to those regions.

Chapter 5 Conclusion and future directions

5.1 Main findings of this doctoral thesis

The diamondback moth (DBM), *Plutella xylostella*, is well known for its extensive adaptation and distribution, genetic polymorphism, and strong resistance to a broad range of insecticides, while knowledge on the genetic basis of these traits remains surprisingly limited. Based on various molecular markers, I uncovered the history of DBM's evolutionary origin and regional distribution in different geographic areas, documented its genetic diversity and variation, and characterized some of its patterns of population expansion and local adaptation. My findings reveal the recent colonization of *P. xylostella* across different parts of the world possibly facilitated by increased human activities.

In Chapter 2, newly isolated microsatellite markers were used to analyze the genetic structure of nine populations across the Taiwan Strait of China (Taiwan and Fujian). A total of 12,152 simple sequence repeats (SSRs) were initially identified from the *P. xylostella* transcriptome (~94 Mb), with an average of 129 SSRs per Mb. Nine SSRs were validated as polymorphic markers, and eight were used for this population genetic study. My data showed that these *P. xylostella* populations could be divided into distinct two clusters, likely due to annual wind patterns in this region. A pattern of isolation by distance among the local populations within Fujian was found, and may be related to transportation of market vegetables. Considering the complexity of *P. xylostella* population genetic structure from local to regional to global levels, I propose that developing ecologically sound strategies for managing this pest will require knowledge of the link between population ecology and its genetic structure.

In Chapter 3, I presented phylogeographical analyses of *P. xylostella* and its dominant parasitoid *Cotesia vestalis* using mitochondrial markers, revealing the evolutionary processes of these two species in East Asia. My data demonstrated that *C. vestalis* adapted to *P. xylostella* as a new host by ecological sorting, as *P. xylostella* expanded its geographical range in East Asia where the parasitoid is posited to originate. Associated with *P. xylostella*'s invasion, *Wolbachia* symbionts were introduced into local populations of the herbivore and parasitoid through inter-specific transfer. This study provides an important basis for better understanding the impacts of

biological invasions on genetic configuration of local species pools, and may help integrated management of invasive pests in the context of global change and human activities. In addition, by showing the evolutionary origin of *C. vestalis*, it is highly recommended that the *C. vestalis*-based classical biological control programs in the future should be conducted by sampling individuals from the region of evolutionary origin, i.e. Southwest China.

In Chapter 4, I explored the patterns of genetic diversity and variation of DBM, and identified its' evolutionary origin and regional distribution, based on SNPs of 174 *P. xylostella* genomes from 38 sites across the North and South American continents. Identification of site(s) of origin of DBM populations invading northern US and Canada and their genetic background, has important implications for developing regional management strategies. Historical information on the insecticide treatment regime used on the migrants at their site of origin is a key factor for determining the appropriate control recommendations for use in northern US and Canada, considering DBM's rapid development of resistance to agrochemicals. With the data obtained from 12 selected countries, I also demonstrated signatures of local adaptation and selection that are associated with insecticide resistance and odour reception. The molecular signature of local adaptation identified in this study not only lists some of the phenotypic differentiation between DBM populations in NA and SA, but also enrich our knowledge of the underlying mechanisms associated with some of the diverged phenotypes. Differentiated frequency of alleles associated with insecticide resistance implies localized regimes of insecticide application. Resistance that might have resulted from certain active ingredients of frequently used insecticide should be one of our top concerns when developing management strategies; even where we don't observe field resistance at present. On one hand, such resistance may be reduced by silence or knockout of the target genes; on the other hand, if the resistance is observed in South American populations, farmers and managers in South America should be advised to try agrochemicals with different modes of action. In addition, one of the potential phenotypes that may have resulted from localized allele frequency is related to odor reception. In terms of management, my recommendation based on this finding is to introduce some of the indigenous *Brassica* species from South America to North America or vice versa, to test if some of those native species can be used as trap crops that can be intercropped with the major cash crops, or can provide extra and better resources than those native species, so as to rescue those local cash crops in a more cost-

effective and environmentally-friendly way.

This overall investigation is well positioned within the rapidly growing area of phylogeography by taking advantage of recent development in molecular biology and population genetics/genomics. Understanding the dynamics of an insect pest outbreak is important from a management perspective. However, knowing the source and dispersal patterns of a pest is even more crucial to help delineate potential boundaries for control and provide the basis for developing strategies to prevent future expansion, especially of insecticide-resistant strains. In the case of *P. xylostella*, it is imperative to have a comprehensive knowledge of its genetic variation, population structures, and expansion patterns over wide spatial scales to efficiently define regional control strategies that will be effective in the long term. This is of even greater importance in the face of global climate change, as overwintering of this species could become more frequent and widespread in temperate regions, especially in North America.

5.2 Future directions

5.2.1 Global phylogeographical study of the diamondback moth

Genome-wide SNPs are recommended to investigate ecological and evolutionary questions by providing molecular evidence with the highest possible resolution. The results presented in this thesis provide an example of how such molecular markers (genome-wide SNPs) may help address ecological questions of importance, such as dispersal and migration, which may not be fully answered by direct observation or examined by analysis using traditional markers. In order to formulate better regional management strategies by considering wider spatial scales based on enriched knowledge of the biology and ecology of a globally-distributed pest such as *P. xylostella*, it is essential to investigate genetic variation and differentiation of *P. xylostella* populations with samples from larger geographical areas, with the goal of uncovering molecular mechanisms associated with insecticide resistance, long-distance migration, and environmental (climatic) adaptation. Phylogeographical study in other parts of the world (beyond the Americas and Parts of Asia) would be expected to provide additional clues to solving the above questions.

5.2.2 Analysis of genes associated with local adaptation

Based on the list of localized resistance-related genes with highly differentiated SNPs that lead to potential changes in protein structure, further work could be conducted to confirm the functions of these mutants, enriching our insights into functions of the identified SNPs associated with local adaptation. Study of interactions between common agrochemicals and target proteins may help verify the degree of variation resulting from localized alteration of protein structure and function. Crystallization of protein adducts with selected insecticides can be performed and studied by X-ray diffraction to understand the adduct formation mechanism at the molecular level. Studies including *in vitro* formation and characterization of protein adduct with insecticides by biophysical techniques like fluorescence and UV spectroscopy can aid in understanding its role in the development of resistance.

5.2.3 Landscape factors shaping *P. xylostella*'s distribution and migration

Natural and human-aided transport is responsible for many contemporary species introductions, invasions, as well as distributions, especially in the case of *P. xylostella*, which can disperse long distances under favorable meteorological conditions, such as air streams. However we have limited information and knowledge about the contributions of these two factors in shaping *P. xylostella* movement and contemporary distribution.

Understanding dispersal dynamics for invasive species can streamline mitigation efforts by targeting routes that contribute disproportionally to spread, but to date, the relative roles of human-aided and natural movement have not been rigorously evaluated. Landscape genetics/genomics studies measuring correlations between landscape distance and genetic distance, and/or modeling landscape effects on genetic distance may help to identify dispersal corridors and inform better management strategies.

In addition, spatial analysis tools are available for combining molecular and environmental data to identify candidate loci for selection. Based on our large volume of SNPs data, the association of allelic frequencies at marker loci with environmental variables can be examined in whole-genome scans, and provide a list of loci that are potentially related to local adaptation.

5.2.4 Phylogeographical study on *Wolbachia*

Wolbachia are maternally-inherited symbiotic bacteria, commonly found in arthropods, that are able to manipulate the reproduction of their hosts in order to maximize their transmission. Study of the coevolution of microbial symbionts and their hosts is of great importance for understanding the impact of microbes on hosts. The evolutionary history of endosymbionts like *Wolbachia* can be revealed by integrating information on infection status in natural populations with patterns of sequence variation in *Wolbachia* and host mitochondrial genomes. Phylogeographical studies of *P. xylostella* and its associated *Wolbachia* strains therefore will enrich our insights into the timing of infection, patterns of transmission (vertical vs. horizontal transmission), degree of spread through populations of interest, and reveal the evolutionary mode and temporal dynamics of the *P. xylostella*-*Wolbachia* symbiosis. With the development of high-throughput sequencing technologies, it is now possible to obtain complete genomic information for microbes and their associated hosts, providing a better understanding of interactions between species and their intracellular symbionts, such as *Wolbachia*.

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