Population structure of the glasshouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), shows multiple introductions to the UK

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Abstract

1 Whiteflies are major pests of many crops worldwide. *Trialeurodes vaporariorum* was introduced into the UK about 160 years ago. We aimed to understand their genetic diversity and population structure, and therewith their invasion history.

2 Mitochondrial CO1 sequencing showed that *T. vaporariorum* had a low level of variation. Microsatellite analysis showed high diversity and indicated two, six and ten clusters, which were, to a limited extent, linked to locations, but not to host plants. The primary symbiont *Portiera aleyrodidarum* was detected in both sexes of *T. vaporariorum*, whereas only one secondary symbiont, *Arsenophonus* sp., was detected in almost all females, but not males.

3 The population structure suggested that glasshouse agroecosystems restricted gene flow between glasshouse whitefly populations and that the movement of glasshouse whitefly was linked to human-assisted spread.

4 Taken together, the results suggested multiple, but limited numbers of introductions of *T. vaporariorum* mainly from countries nearest to UK.

Keywords: genetic diversity, microsatellite, mitochondrial haplotype, mtCOI, population structure, endosymbionts.

1 Introduction

Whiteflies (Hemiptera: Aleyrodidae) are considered major pests of many crops worldwide. More than 1450 species have been described in two subfamilies, and the most economically significant pest species are members of the Aleyrodinae (Bink-Moenen, 1990; Martin *et al.*, 2000). The most important and serious whitefly species are the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), and the glasshouse whitefly, *Trialeurodes vaporariorum* (Westwood). They are considered key insect pests of many vegetable and ornamental crops, causing damage to plants either directly by feeding or indirectly by transmitting viruses (CABI, 2018). Whiteflies typically have short life cycles, which are dependent on climatic conditions, especially temperature. In normal conditions they produce several generations every year (Martin *et al.*, 2000). The rapid population growth of whiteflies is due in part to arrhenotokous parthenogenesis, in which non-fertilized eggs grow into males and fertilized eggs into females.

Often within insect pest species a complex of biotypes can be described on the basis of insecticide resistance, morphology, behaviour and/or DNA sequence of the mitochondrial cytochrome oxidase I (mtCOI) gene. For example, recent research on *B. tabaci* has concluded that the species is a complex with more than thirty-one relevant biotypes (Boykin *et al.*, 2012; Boykin *et al.*, 2013), while in *T. vaporariorum* nineteen mtCOI haplotypes have been detected in the global population of this species (Prijovic *et al.*, 2014). The genetic complexity of whitefly species is important for several reasons, including the development of insecticide resistance in response to selection pressure, host differences, and geography, all of which might impact on their vector potential for various viruses (Bird, 1957; Mound, 1963; Costa and Russell, 1975; Bird and Maramorosch, 1978). Those strains of whitefly developing insecticide resistance pose a problem for future management (Gorman *et al.*, 2002; Karatolos *et al.*, 2010; Pappas *et al.*, 2013). In comparison to *B. tabaci*, fewer studies on population

structure and phylogeny have been carried out with *T. vaporariorum*. One study used mtCOI sequencing and nuclear markers to assess the genetic diversity and phylogenetic structure of glasshouse whitefly in India (Roopa *et al.*, 2012), indicating low mtCOI and nuclear marker diversity and differentiation between populations. These findings have been confirmed by Kapantaidaki *et al.* (2015) and Prijovic *et al.* (2014) who assessed glasshouse whitefly populations from Europe and the USA. Furthermore, population genetic structure of non-native *T. vaporariorum* has been studied in different regions and habitats of Finland, Greece, and China (Gao *et al.*, 2014; Ovcarenko *et al.*, 2014).

Biological invasions and introduction of non-native species may be introductions by human or by-products of human travel and trade activities and both have important implications for biodiversity and agriculture (Pimentel *et al.*, 2001; Riis and Nachman, 2006). Recording of alien species relies on chance observation and survey. Therefore, the introduction and distribution pathways of aliens often go unnoticed, especially when introduction follows failures in quarantine at borders (Estoup and Guillemaud, 2010). Research on the population structure and genetic diversity of species, when introduced in a non-native area, can assist in explaining the origin, routes and times of introduction (Lombaert *et al.*, 2014) and provide details essential for management and the avoidance of future non-native introduction (Signorile *et al.*, 2014). Various introductions, secondary expansions of introduced populations and management efforts can both lead to structured populations in introduced regions (Berthouly-Salazar *et al.*, 2013; Cao *et al.*, 2016), while gene flow among introduced populations ultimately decreases levels of population differentiation (Tsuchida *et al.*, 2014), except at loci with selection. However, no study has yet assessed the genetic structure and introduction of glasshouse whitefly in the UK since the species established here, about 160 years ago.

Obligate and facultative, maternally inherited endosymbionts are common and important components of terrestrial arthropod biology and ecology (Cass *et al.*, 2014). Whiteflies are known to host the obligatory primary symbiont *Portiera aleyrodidarum*, which has a long coevolutionary history with all members of the Aleyrodinae subfamily (Thao and Baumann, 2004). *Portiera spp* are known to supplement the hosts' diet with essential nutrients, such as amino acids, as well as with carotenoids that present significant anti-oxidant action (Santos-Garcia *et al.*, 2012). Further to the primary endosymbiont, whiteflies contain a range of secondary symbionts, including species of *Candidatus, Hamiltonella, Cardinium* (Bacteroidetes), *Fritschea, Wolbachia*, *Arsenophonus* and *Rickettsia* (Rickettsiales) (Zchori-Fein and Brown, 2002; Nirgianaki *et al.*, 2003). Bacterial symbionts have been reported to have effects on aspects of host biology, including genetic diversity, nutrition, survival, reproduction, insecticide resistance, and the ability to cope with environmental factors (Saridaki and Bourtzis, 2010; Kikuchi *et al.*, 2012) . Both the symbionts and mtDNA are vertically transmitted and linked to the evolutionary history of the insect host, and thus may be used to shed light on evolutionary and demographic processes relating to both sides of the symbiosis (Hurst and Jiggins, 2005; Werren *et al.*, 2008).

Here we present the first extensive genetic data on the population structure of *T. vaporariorum* in the UK and investigate their endosymbiont prevalence and distribution. We posed two questions: Have many introductions occurred? Do the new habitat and other agricultural applications structure the pest populations? The answers may improve our understanding of gene flow and patterns of population genetic structure and the factors that affect this.

2 Experimental procedures

2.1 Field sampling

Adults of glasshouse whitefly were collected from tomato, cucumber, and ornamental crops from commercial glasshouses in 12 locations throughout the UK during summer and autumn, and some locations were sampled in both 2014 and 2015 (Table 1). In addition, a laboratory colony was used; this was taken from a mixed-age colony maintained at Newcastle University (UK) on aubergine (*Solanum melongena*). This colony was obtained from Rothamsted Research, and originally collected in 1960 in Kent from French bean plants (B. Brogan pers. comm.). At least 20 adult whitefly specimens were collected from whitefly-colonized plants at each location. A total of 400 individuals from 20 populations (20 individuals per population) were genotyped (Table 1). The whitefly specimens were stored in 95% ethanol at −20 °C until DNA was extracted.

2.2 Confirming the identity of specimens morphologically

Specimens were slide-mounted with Canada balsam using the procedure described by Brown (1997). The taxonomic characters used for identification were based on Hill (1969). Three specimens were collected from each population in order to confirm identification. The characteristics of our samples were assessed with regard to compound eyes, forewings, mesothoracic legs, antennae, external genital organs and the abdomen.

2.3 MtCOI sequencing

Since whiteflies are a haplodiploid species, only the total genomic DNA (gDNA) of adult females was extracted, as described in Tsagkarakou *et al.* (2007), by placing them in a 1.5mL Eppendorf and grinding with a pestle in 50mL of ice-cold lysis buffer (100mM NaCl, 10mM Tris-HCL, pH8.0) containing 0.4mg/mL^{-1} of proteinase K. The extracts were incubated at 55 °C for 1h and at 85 °C for 5 min prior to a 5 min centrifugation (13,000 g) to pellet debris. The supernatant was used as the DNA source for the polymerase chain reaction (PCR).

At least four individuals of *T. vaporariorum* for each location and both years from each site, in total 96, were sequenced. The PCR of mitochondrial cytochrome c oxidase I (mtCOI)

was performed using specific primers for this region, CO1-F: 5'-GCCTGGTTTTGGCATTA-3', and CO1-R: 5' GCTTATTTAGCACCCACTCTA-3'), which produced a ~752bp product (Gao *et al.,* 2014). PCR reactions were carried out in a 10μL volume containing 1μL DNA template, 0.5μL of each primer, 2μL 5× PCR MyTaq reaction buffer, and 0.5 units MyTaq DNA polymerase (Bioline). The PCR products were visualized on a 2% agarose gel containing ethidium bromide. The PCR products were purified using Exo-Sap. The following amounts of reagents were added into each sample: 1.5 μL of Shrimp Alkaline Phosphatase (SAP), 2.02 μL of Shrimp Alkaline Phosphatase buffer and 0.22 μL of Exonuclease I. The reactions were incubated in a thermocycler at 37 °C for 40 minutes and 80 °C for 15 minutes.

The purified PCR products were sequenced using ABI Prism BigDye® Terminator Version 3.1 Cycle Sequencing Kits (Applied Biosystem, Foster City, California, USA). Sequencing reactions were performed in 10 μL containing 1.5 μL of 5X sequencing buffer, 0.5 pmol of COI forward primer, 1 μL of BigDye terminator sequencing mix and 1 μL of purified PCR product. The reactions were amplified by 35 cycles of 96 °C for 10 s, 52 °C for 5 s and 60 °C for 4 min. Sequencing products were purified with ethanol precipitation. The sequences were visualized on a 3130XL Genetic Analyzer (Applied Biosystems). All individuals were sequenced in one direction only. The sequences were checked manually and aligned using Geneious, version 6.1.4 (Kearse *et al.*, 2012), and compared with those available in Genbank using the BLAST algorithm of the National Center for Biotechnology Information (NCBI).

2.4 Molecular identification of endosymbionts

To detect the presence of primary and secondary bacterial symbionts, PCR with DNA from both whitefly sexes was performed using species specific primers for the 16S rRNA genes in *Portiera* sp. *Wolbachia, Rickettsia*, *Hamiltonella,* and *Cardinium* and the 23S rRNA genes in *Arsenophonus* and *Fritschea,* as described in Kapantaidaki *et al.* (2015). PCR amplification

was as described above for the mtCO1 sequencing. In order to check the quality of DNA extraction, samples that tested negative for all endosymbionts were cross-checked for the obligate endosymbiont *P. aleyrodidarum* using primers (518F and 799r) for the 16S rRNA gene to check the DNA quality (Muyzer *et al.*, 1993; Chelius and Triplett, 2001; Zchori-Fein and Brown, 2002). In addition, we included adults of *B. tabaci* to test for the reliability of secondary symbionts PCR testing.

The PCR reaction had the following conditions: initial denaturation at 93 °C for 2 min, followed by 35 cycles of 93 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The PCR products were visualized on a 2% agarose gel containing ethidium bromide. The PCR products shown on gel were purified using Exo-Sap, and sequencing was performed and visualized on a 3130XL Genetic Analyzer as described above in mtCO1 sequencing. The sequences obtained were manually edited using Geneious, version 6.1.4 (Kearse *et al.*, 2012). All sequences were compared with those in the GenBank database using the NCBI BLAST algorithm.

2.5 Microsatellite genotyping

Nine microsatellite primers pairs (Tvap-3-3, Tvap-1-4, Tvap-1-5, Tvap-1-1C, Tvap-1-2, Tvap-3-1, Tvap-2-2C, Tvap-3-2, and Tvap-4-2), as described in Ovcarenko *et al.* (2013), were used to amplify microsatellite loci using *T. vaporariorum* DNA as the template. Four hundred females from 20 populations were assessed for three sets of multiplex amplification reactions: set 1 (Tvap-3-3, Tvap-1-4, and Tvap-1-5); set 2 (Tvap-1-1C, Tvap-1-2, and Tvap-3-1); and set 3 (Tvap-2-2C, Tvap-3-2, and Tvap-4-2). The PCR amplification was performed in 10μL containing 5 ng DNA, 2μL 5× PCR reaction buffer, 2 mM $MgCl_2$, 0.2 μM dNTPs, 0.2–0.4 μM of each primer, and 0.5 units MyTaq DNA polymerase (Bioline). PCR reactions were run in conditions of initial denaturation at 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The PCR products were separated on a

3130XL Genetic Analyzer and the allele size was determined using GeneScan™ ROX500 size standard, using GeneMapper software version 3.2 (Applied Biosystems) and were confirmed manually.

2.6 Data analysis of population genetic structure and genetic diversity

For each of the 20 populations of *T. vaporariorum*, the average number of alleles per locus (N_a), effective number of alleles (A_e), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using GenAlEx v. 6.5 (Peakall and Smouse, 2012). The difference in H_e between the populations was statistically analysed using the Fisher Least Significant Difference (LSD) Test in Minitab® v17 software (2013 Minitab Inc.). Weir and Cockerham's estimator of the fixation index *F* (Weir and Cockerham, 1984) was calculated and tested for significance from zero using GENEPOP v.3.4, with zero meaning random mating (Raymond and Rousset, 1995). The program FSTAT 2.9.3.2 was used to calculate allelic richness (A_r) (Franks *et al.*, 2011). The population differentiation approach was based on F_{st} values calculated using GENEPOP v.3.4 (Raymond and Rousset, 1995). The distribution of genetic variation was investigated by performing an analysis of molecular variance (AMOVA) and a Principal Coordinate Analysis (PCoA) both were carried out using GenAlEx v. 6.5.

The genetic clustering of samples was examined using STRUCTURE v.2.3.2 (Pritchard *et al.*, 2000) with a burn-in of 150,000 iterations and 500,000 Markov chain Monte Carlo (MCMC) repetitions under the no admixture ancestry model and using prior allele frequency information. Twenty independent runs were performed for each K value, ranging from K = 1 to 21, and ΔK was used to calculate the optimal number of genetic clusters (K) using Structure Harvester (Earl and Vonholdt, 2012). The results were combined and visualised using online POPHELPER software (Francis, 2017).

3 Results

3.1 Species identification and mtCOI sequencing

Morphological examination confirmed that the species was *T. vaporariorum*. Ninety-one out of the 96 UK individuals had a mitochondrial COI sequence belonging to haplotype mtH1, with sequencing length ranging from 626 to 657 bp. Interestingly, we found five mitochondrial haplotype mtH3, which is new for UK records, with sequencing lengths 530 and 525 bp, with three in Essex and two in Norfolk, respectively. The mtH1 and mtH3 are described by Prijovic *et al.* (2014) in the NCBI dataset, and differ from each other by a single nucleotide C-T substitution, at position 154. Two mitochondrial haplotype mtH3 and one mtH1 sequences were deposited in Genbank with accession numbers KX679578, KY048293, and KX679581, respectively.

3.2 Genetic diversity

All populations showed high genetic diversity (Table 1). The average number of alleles per locus (N_a) ranged from 2 (Lab colony, LC15) to 3.556 (Dundee, DU14), and the effective number of alleles (N_e) ranged from 1.718 (Lab colony, LC15) to 2.457 (Orkney, Or14). H_o in most populations was higher than H_e. The expected heterozygosity (H_e) ranged from 0.366 (Lab colony, LC15) to 0.576 (Orkney, Or14), while the observed heterozygosity (H_o) ranged from 0.450 (Lab colony, LC15) to 0.683 (Orkney, Or14). There was a significant difference ($P < 0.05$) between populations for their genetic diversity, expressed as H_e, with higher diversity in *T. vaporariorum* populations from Or14, DU14, NO14, and ES14, than in the lab colony LC15. The population from Orkney (Or14) had the highest value of H_e, at 0.576, while the Lab colony (LC15) had the lowest value at 0.366. The populations from Scotland (Orkney/Or14; Dundee, DU14), East York (EYO14), Essex (ES14), and Norfolk (NO3_14) exhibited a H_e value higher than the mean value of 0.478.

In terms of genetic structure between populations, 97.5% of pairwise F_{st} comparisons (195 out 200) were significantly different from zero (Table 2). Only geographically close populations or those from the same location did, in some cases, not have a significant differentiation; for example, among the Herefordshire (HE2_14-HE4_14) samples. Estimates of pairwise F_{st} values ranged from 0.02 (HE4_14/HE2_14) to 0.33 (LC15/ERYS14). The AMOVA revealed genetic differentiation among populations, explaining 17% of the total genetic variance, while the remainder of the variation (83%) was within populations.

3.3 Symbionts

Ten females and ten males from each population were used to screen for primary and six secondary symbionts. The primary symbiont *P. aleyrodidarum* was identified in all samples of both sexes, also indicating that our DNA extracts were of good quality. The infection status of *T. vaporariorum* was 96.6% for one secondary symbiont *Arsenophonus* sp., in the females with none in the males, while no PCR products were found for the other symbionts (Table 3). The PCR products were sequenced to confirm the genus and species of symbiotic bacteria using NCBI databases. The *Arsenophonus* sp. 23S rRNA sequencing length was 447 bp, whereas the primary endosymbiont *P. aleyrodidarum* 16S rRNA sequence was 784 bp in length. Single sequences of a primary and a secondary endosymbiont were deposited in Genbank under accession numbers KY243936 and KY457224.

3.4 Microsatellite genotyping

The PCoA approach showed some grouping of individuals by population (Suppl. Material, Fig 1), with 32.03 % of total variation explained by the first two axes (11.90% and 22.91%, respectively). For example, HE2_14-H4_14 and HE15 populations are presented on the right, while Orkney (Or14 and Or15) populations are presented on the left in the figure.

The clustering method implemented in STRUCTURE determined three optimal groupings of *T. vaporariorum* individuals (Fig 1) with two, six and ten genetic clusters, as indicated by

high value of Delta K (ΔK) against K (Suppl. Material, Fig 2). Thus, the groupings at these K values were examined. K = 2 revealed seemingly random clusters of populations from the UK (Fig 1). K = 6 and 10 gave some information based on geographical patterns, visualized by their proportional Q values (Fig 2). In some cases samples from the same location, but different years grouped together, in other cases they do not. For example, samples from Herefordshire (HE2_14, HE3_14, HE4_14, and HE15) grouped. Samples from Dundee (DU15 and DU14), partially share STRUCTURE groupings. On the other hand, samples from the same place did not group together for Orkney (Or14, Or15), Norfolk (NO14, NO15), and East Riding Yorkshire (ERYS15, ERYS14), despite coming from the same host plant. There is no overall effect of host plant on genetic clustering of this pest (Fig 1).

4 Discussion

This study presents, for the first time, extensive data on the population structure and genetic diversity of *T. vaporariorum* in the UK, collected from 12 locations and different host plants over two years. The results show high diversity using microsatellite markers, but limited diversity at the mitochondrial level and only one secondary symbiont species associated with *T. vaporariorum*.

Most of the mtDNA COI sequences (91) of *T. vaporariorum* individuals present in the UK belonged to mitochondrial haplotype mtH1, which is also common in the Netherlands and France (Malumphy *et al.*, 2007; Kapantaidaki *et al.*, 2015). Haplotype mtH3 was recorded in the UK for the first time, namely in Essex and Norfolk, in the southeast of England. The mtH3 is most common in the USA and was also previously recorded in Spain on the Canary Islands and in Serbia (Malumphy *et al.*, 2007; Prijovic *et al.*, 2014). The likely recent introduction of mtH3 in the UK might be a result of human activities, e.g. plant import from Europe or the USA. In Serbia and its neighbouring European countries, six mitochondrial haplotypes of glasshouse whitefly, including mtH1 and mtH3, have been

recorded, while nineteen mtCOI haplotypes have been detected in the global population of this species (Prijovic *et al.*, 2014). Our low level of variation in mtCOI sequences of *T. vaporariorum* populations was similar to the results of Kapantaidaki *et al.* (2015), who found that most mtCOI sequences collected from the USA and some European countries belonged to haplotype mtH1 and a single individual to mtH3. A similar level of genetic variation has been found for this species in Serbia and its neighbouring countries (Prijovic *et al.*, 2014). The likely explanation for the lack of COI sequence variation in our data is the recent introduction of this species in the UK, around 160 years ago (Mound and Halsey, 1978). This means that there was probably not enough time for the evolution of new haplotypes in the UK, and variation of *T. vaporariorum* must have arisen through imports.

The facultative symbiont *Arsenophonus* sp. has been shown to be a reproductive manipulator in other insect species (Werren *et al.*, 1986; Balas *et al.*, 1996). *T. vaporariorum* populations from the UK harboured just one secondary symbiont. *Arsenophonus* sp. was prevalent in 96.6% of female glasshouse whitefly, meaning near-fixation in the UK, but was absent in males. Symbionts, including *Arsenophonus* sp., are known to affect reproduction and are often specifically known as 'male killers' (Gherna *et al.*, 1991; Ferree *et al.*, 2008; Duron *et al.*, 2010). Therefore, *Arsenophonus* sp. could be killing males in the UK *T. vaporariorum* populations, but further investigation is needed to confirm this. It would be possible to conduct laboratory experiments for *T. vaporariorum* with females having or not having the symbionts *Arsenophonus* sp. and then to observe the sex ratios of their offspring to confirm the male-killing function of this secondary symbiont.

In general, infection with primary and secondary symbionts reaching fixation, or nearly fixation, suggests that the symbionts play an important role, a mutualistic relationship, with their insect host. We are confident about our results regarding the absence of other secondary symbionts because all DNA samples tested showed the presence of the primary

303 symbionts. Also, we used the same methods as described by Kapantaidaki *et al.* (2015), who
304 successfully showed presence of other secondary symbionts associated with *T. vaporariorum*.
305 Lastly, we also tested the same method on DNA extracted from *B. tabaci* Middle East-Asia
306 Minor1 (MEAM1). This showed the same primary, but different secondary symbionts;
307 namely, *Hamiltonella* sp. and *Rickettsia* sp. (Kareem et al, results unpubl.). In addition, our
308 results are similar to those of another study that showed that males of *T. vaporariorum* from
309 Japan did not harbour *Arsenophonus* sp. despite the fact that females from this population
310 were all infected (Kapantaidaki *et al.*, 2015). However, populations from Croatia, Bosnia, and
311 Herzegovina harboured both *Arsenophonus* sp. and *Hamiltonella* sp. bacterial symbionts in
312 both males and females (Skaljac *et al.*, 2010; Skaljac *et al.*, 2013). An even more diverse
313 community of bacterial symbionts was recorded in *T. vaporariorum* populations from
314 Montenegro, where the populations harboured *Rickettsia, Hamiltonella, Arsenophonus,*
315 *Wolbachia* and *Cardinium* (Prijovic *et al.*, 2014). Lack of diversity of secondary symbionts in
316 our results might indicate limited introduction of insects and/or introduction from regions
317 only having *Arsenophonus* sp. as secondary symbionts.
318 UK populations of whitefly collected from glasshouses exhibit significant genetic
319 diversity. The mean value of H_e in UK *T. vaporariorum* populations (0.477) was similar to
320 those in China, Greece, and Finland (0.368, 0.459 and 0.443), respectively. A lower genetic
321 diversity was indicated in the laboratory colony, which might be explained by selection
322 and/or a prolonged bottleneck (Hadjistylli *et al.*, 2016). Most of our samples were from
323 commercial glasshouses, which are often characterized by intense management compared to
324 field crops (Ovcarenko *et al.*, 2014). Enclosure in glasshouses affects the genetic diversity of
325 insects by the restriction of gene flow (Hoffmann and Willi, 2008). Crop management
326 applications and regular population management can cause population bottlenecks, leading to
327 potentially strong effects of random genetic drift and decreases in heterozygosity, as well as

increasing population genetic differentiation (Tsagkarakou *et al.*, 1998). Therefore, the genetic clustering of *T. vaporariorum* based on microsatellites showed some structure of populations in the UK in some cases related to geography, but not related to host plant. Similarly, structural results based on geographical patterns were also indicated in glasshouse whitefly populations in China, Finland, and Greece (Gao *et al.*, 2014; Ovcarenko *et al.*, 2014). The results of STRUCTURE are supported by significant F_{st} values indicating genetic differentiation between populations. In addition, in some cases STRUCTURE showed different clusters for two years of collections from the same location. This means that some growers were able to eradicate whitefly from previous seasons, presumably through sanitising the glasshouse and the plants, and new infestations were brought with new crops. However, other growers maintained whitefly from one season into another.

The genetic diversity and structure of invasive species has been studied in various insect taxa. For example, genetic diversity patterns involving multiple introductions has been demonstrated in sweet potato whitefly *B. tabaci* and thrips *Frankliniella occidentalis* (Delatte *et al.*, 2006; Cao *et al.*, 2017). Multiple introductions of invasive species are regarded as the main source of genetic variation (Reem *et al.*, 2013), which is often associated with more successful invasions for some species (Roman and Darling, 2007; Suarez and Tsutsui, 2008). The low level of genetic variation of mtCO1 could be a consequence of extensive insect control measures that include biological control, and use of insecticides to reduce and/or eradicate *T. vaporariorum*. However, considerable diversity at the nuclear level, but little or no diversity at the cytoplasmic level (mtDNA and symbiont) makes it likely that multiple introductions from the same region occurred. Extensive sampling of populations, particularly from the west and north of the UK, is needed to confirm the low level of mtDNA, which

might help extend our understanding of the biology, ecology and spread of this damaging and invasive insect pest.

5 Conclusion

Populations of *T. vaporariorum* in the UK exhibit genetic differentiation, demonstrating the possibility that multiple introductions of *T. vaporariorum* into the UK have occurred. The results showed some structure of populations, with clustering by geographical location and not by crops. All *T. vaporariorum* individuals in the north and midlands of the UK belong to mtH1while mtH3 has been recorded in the UK for the first time in the south east of England, indicating that there have been at least two introductions. Tests revealed the presence of *Portiera* sp., an obligate endosymbiont, in both sexes, whereas the facultative symbiont *Arsenophonus* sp. was detected in females only. The glasshouse agroecosystem and repeated imports from a limited region may have contributed to variation at nuclear, but not at cytoplasmic level. This has likely contributed to population genetic structure through restricting gene flow between locations.

6 Acknowledgments

We would like to acknowledge the UK sample providers, the sponsors, the Ministry of Higher Education and Scientific Research in Iraq, and the Agriculture College at the University of Karbala for their funding for Ali Kareem, and Dr Samuel Logan for laboratory assistance.

References:

Balas, M.T., Lee, M.H. and Werren, J.H. (1996) 'Distribution and fitness effects of the son-killer bacterium in *Nasonia*', *Evolutionary Ecology*, 10(6), pp. 593-607.

Berthouly-Salazar, C., Hui, C., Blackburn, T.M., Gaboriaud, C., van Rensburg, B.J., van Vuuren, B.J. and Le Roux, J.J. (2013) 'Long-distance dispersal maximizes evolutionary potential during rapid geographic range expansion', *Molecular Ecology*, 22(23), pp. 5793-5804.

Bink-Moenen, R.M.a.M., L. A. (1990) 'Whiteflies: diversity, biosystematics and evolutionary patterns', in *Whiteflies: their bionomics, pest status and management. Ed. D. Gerling*. Andover: Intercept, pp. 1-12.

Bird, J. (1957) 'A whitefly transmitted mosaic *Jatropha gossypifolia* ,' *Technical Paper*, 22, pp. 1-35.

Bird, J. and Maramorosch, K. (1978) 'Viruses and virus diseases associated with whiteflies', *Advances in Virus Research*, 22, pp. 55-110.

Boykin, L.M., Armstrong, K.F., Kubatko, L. and De Barro, P. (2012) 'Species delimitation and global biosecurity', *Evolutionary Bioinformatics Online*, 8(8), pp. 1-37.

Boykin, L.M., Bell, C.D., Evans, G., Small, I. and De Barro, P.J. (2013) 'Is agriculture driving the diversification of the *Bemisia tabaci* species complex (Hemiptera: Sternorrhyncha: Aleyrodidae)?: Dating, diversification and biogeographic evidence revealed', *BMC Evolutionary Biology*, 13(1), p. 228.

Brown, P.A. (1997) *A review of techniques used in the preparation, curation and conservation of microscope slides at the Natural History Museum, London*. Biology Curator's Group.

CABI 2018 (2018) '*Trialeurodes vaporariorum*: Invasive species compendium'. Wallingford, UK: CAB International 05 April 2018. Available at: https://www.cabi.org/isc/datasheet/54660.

Cao, L.J., Wang, Z.H., Gong, Y.J., Zhu, L., Hoffmann, A.A. and Wei, S.J. (2017) 'Low genetic diversity but strong population structure reflects multiple introductions of western flower thrips (Thysanoptera: Thripidae) into China followed by human-mediated spread', *Evolutionary Applications*, 10(4), pp. 391-401.

Cao, L.J., Wei, S.J., Hoffmann, A.A., Wen, J.B. and Chen, M. (2016) 'Rapid genetic structuring of populations of the invasive fall webworm in relation to spatial expansion and control campaigns', *Diversity and Distributions*, 22(12), pp. 1276-1287.

Cass, B.N., Mozes-Daube, N., Iasur-Kruh, L., Bondy, E.C., Kelly, S.E., Hunter, M.S. and Zchori-Fein, E. (2014) 'Bacterial endosymbionts in field-collected samples of *Trialeurodes sp. nr.* abutiloneus (Hemiptera: Aleyrodidae)', *Research in Microbiology*, 165(2), pp. 77-81.

Chelius, M.K. and Triplett, E.W. (2001) 'The diversity of archaea and bacteria in association with the roots of *Zea mays* L.', *Microbial Ecology*, 41(3), pp. 252-263.

Costa, A.S. and Russell, L.M. (1975) 'Failure of *Bemisia tabaci* to breed on cassava plants in Brazil (Homoptera: Aleyrodidae)', *Ciencia E Cultura*, 27(4), pp. 388-390.

Delatte, H., David, P., Granier, M., Lett, J.M., Goldbach, R., Peterschmitt, M. and Reynaud, B. (2006) 'Microsatellites reveal extensive geographical, ecological and genetic contacts

between invasive and indigenous whitefly biotypes in an insular environment', *Genetical Research*, 87(2), pp. 109-124.

Duron, O., Wilkes, T.E. and Hurst, G.D. (2010) 'Interspecific transmission of a male-killing bacterium on an ecological timescale', *Ecology Letters*, 13(9), pp. 1139-1148.

Earl, D.A. and Vonholdt, B.M. (2012) 'STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method', *Conservation Genetics Resources*, 4(2), pp. 359-361.

Estoup, A. and Guillemaud, T. (2010) 'Reconstructing routes of invasion using genetic data: why, how and so what?', *Molecular Ecology*, 19(19), pp. 4113-4130.

Ferree, P.M., Avery, A., Azpurua, J., Wilkes, T. and Werren, J.H. (2008) 'A bacterium targets maternally inherited centrosomes to kill males in *Nasonia*', *Current Biology*, 18(18), pp. 1409-1414.

Francis, R.M. (2017) 'Pophelper: an R package and web app to analyse and visualize population structure', *Molecular Ecology Resources*, 17(1), pp. 27-32.

Franks, S.J., Pratt, P.D. and Tsutsui, N.D. (2011) 'The genetic consequences of a demographic bottleneck in an introduced biological control insect', *Conservation Genetics*, 12(1), pp. 201-211.

Gao, R.R., Zhang, W.P., Wu, H.T., Zhang, R.M., Zhou, H.X., Pan, H.P., Zhang, Y.J., Brown, J.K. and Chu, D. (2014) 'Population structure of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), an invasive species from the Americas, 60 years after invading China', *International Journal of Molecular Sciences*, 15(8), pp. 13514-13528.

Gherna, R.L., Werren, J.H., Weisburg, W., Cote, R., Woese, C.R., Mandelco, L. and Brenner, D.J. (1991) '*Arsenophonus nasoniae* gen. nov., sp. nov., the causative agent of the son-killer trait in the parasitic wasp *Nasonia vitripennis*', *International Journal of Systematic and Evolutionary Microbiology*, 41(4), pp. 563-565.

Gorman, K., Hewitt, F., Denholm, I. and Devine, G.J. (2002) 'New developments in insecticide resistance in the glasshouse whitefly (Trialeurodes vaporariorum) and the two‐spotted spider mite (Tetranychus urticae) in the UK', *Pest management science*, 58(2), pp. 123-130.

Hadjistylli, M., Roderick, G.K. and Brown, J.K. (2016) 'Global population structure of a worldwide pest and virus vector: genetic diversity and population history of the *Bemisia tabaci* sibling species group', *PLoS One*, 11(11), p. e0165105.

Hill, B.G. (1969) 'A morphological comparison between two species of whitefly, *Trialeurodes vaporariorum* (Westw.) and *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) which occur on tobacco in the Transvaal', *Phytophylactica*, 1(3_4), pp. 127-146.

Hoffmann, A.A. and Willi, Y. (2008) 'Detecting genetic responses to environmental change', *Nature Reviews Genetics*, 9(6), pp. 421-432.

Hurst, G.D. and Jiggins, F.M. (2005) 'Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts', *Proceedings of the Royal Society of London B: Biological Sciences*, 272(1572), pp. 1525-1534.

Kapantaidaki, D.E., Ovcarenko, I., Fytrou, N., Knott, K.E., Bourtzis, K. and Tsagkarakou, A. (2015) 'Low levels of mitochondrial DNA and symbiont diversity in the worldwide

agricultural pest, the greenhouse whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)', *Journal of Heredity*, 106(1), pp. 80-92.

Karatolos, N., Denholm, I., Williamson, M., Nauen, R. and Gorman, K. (2010) 'Incidence and characterisation of resistance to neonicotinoid insecticides and pymetrozine in the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae)', *Pest Management Science*, 66(12), pp. 1304-1307.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S. and Duran, C. (2012) 'Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data', *Bioinformatics*, 28(12), pp. 1647-1649.

Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K. and Fukatsu, T. (2012) 'Symbiont-mediated insecticide resistance', *Proceedings of the National Academy of Sciences of the United States of America*, 109(22), pp. 8618-8622.

Lombaert, E., Guillemaud, T., Lundgren, J., Koch, R., Facon, B., Grez, A., Loomans, A., Malausa, T., Nedved, O. and Rhule, E. (2014) 'Complementarity of statistical treatments to reconstruct worldwide routes of invasion: the case of the Asian ladybird *Harmonia axyridis*', *Molecular Ecology*, 23(24), pp. 5979-5997.

Malumphy, C., Suarez, M.B., Glover, R., Boonham, N. and Collins, D.W. (2007) 'Morphological and molecular evidence supporting the validity of *Trialeurodes lauri* and *T. ricini* (Hemiptera : Sternorrhyncha : Aleyrodidae)', *European Journal of Entomology*, 104(2), pp. 295-301.

Martin, J.H., Mifsud, D. and Rapisarda, C. (2000) 'The whiteflies (Hemiptera : Aleyrodidae) of Europe and the Mediterranean basin', *Bulletin of Entomological Research*, 90(5), pp. 407-448.

Mound, L.A. (1963) 'Host‐correlated variation in *Bemisia tabaci* (Gennadius)(Homoptera: Aleyrodidae)', *Physiological Entomology*, 38(10‐12), pp. 171-180.

Mound, L.A. and Halsey, S.H. (1978) *Whitefly of the World. A Systematic Catalogue of the Aleyrodidae (Homoptera) with Host Plant and Natural Enemy Data*. UK: John Wiley and Sons.

Muyzer, G., De Waal, E.C. and Uitterlinden, A.G. (1993) 'Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA', *Applied and Environmental Microbiology*, 59(3), pp. 695-700.

Nirgianaki, A., Banks, G.K., Frohlich, D.R., Veneti, Z., Braig, H.R., Miller, T.A., Bedford, I.D., Markham, P.G., Savakis, C. and Bourtzis, K. (2003) '*Wolbachia* infections of the whitefly *Bemisia tabaci*', *Current Microbiology* 47(2), pp. 93-101.

Ovcarenko, I., Clouet, C., Knott, E., Tsagkarakou, A. and Gauthier, N. (2013) 'Thirteen polymorphic microsatellate loci and PCR multiplexing in the greenhouse whitefly , *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae)', *Molecular Ecology Resources*, 13(2), pp. 341-343.

Ovcarenko, I., Kapantaidaki, D.E., Lindstrom, L., Gauthier, N., Tsagkarakou, A., Knott, K.E. and Vanninen, I. (2014) 'Agroecosystems shape population genetic structure of the greenhouse whitefly in Northern and Southern Europe', *BMC Evolutionary Biology*, 14(1), p. 165.

Pappas, M.L., Migkou, F. and Broufas, G.D. (2013) 'Incidence of resistance to neonicotinoid insecticides in greenhouse populations of the whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) from Greece', *Applied Entomology and Zoology*, 48(3), pp. 373-378.

Peakall, R. and Smouse, P.E. (2012) 'GenAlEx 6.5: genetic analysis in Excel, population genetic software for teaching and research--an update', *Bioinformatics*, 28(19), pp. 2537-2539.

Pimentel, D., McNair, S., Janecka, J., Wightman, J., Simmonds, C., O'Connell, C., Wong, E., Russel, L., Zern, J., Aquino, T. and Tsomondo, T. (2001) 'Economic and environmental threats of alien plant, animal, and microbe invasions', *Agriculture Ecosystems and Environment*, 84(1), pp. 1-20.

Prijovic, M., Skaljac, M., Drobnjakovic, T., Zanic, K., Peric, P., Marcic, D. and Puizina, J. (2014) 'Genetic variation of the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), among populations from Serbia and neighbouring countries, as inferred from COI sequence variability', *Bulletin of Entomological Research*, 104(3), pp. 357-366.

Pritchard, J.K., Stephens, M. and Donnelly, P. (2000) 'Inference of population structure using multilocus genotype data', *Genetics*, 155(2), pp. 945-959.

Raymond, M. and Rousset, F. (1995) 'Genepop (Version-1.2): Population-genetics software for exact tests and ecumenicism', *Journal of Heredity*, 86(3), pp. 248-249.

Reem, E., Douek, J., Katzir, G. and Rinkevich, B. (2013) 'Long-term population genetic structure of an invasive urochordate: the ascidian *Botryllus schlosseri*', *Biological Invasions*, 15(1), pp. 225-241.

Riis, L. and Nachman, G. (2006) 'Migration, trapping and local dynamics of whiteflies (Homoptera: Aleyrodidae)', *Agricultural and Forest Entomology*, 8(3), pp. 233-241.

Roman, J. and Darling, J.A. (2007) 'Paradox lost: genetic diversity and the success of aquatic invasions', *Trends in Ecology & Evolution*, 22(9), pp. 454-464.

Roopa, H.K., Kumar, N.K.K., Asokan, R., Rebijith, K.B., Mahmood, R. and Verghese, A. (2012) 'Phylogenetic analysis of *Trialeurodes spp*. (Hemiptera: Aleyrodidae) from India based on differences in mitochondrial and nuclear DNA', *Florida Entomologist*, 95(4), pp. 1086-1094.

Santos-Garcia, D., Farnier, P.A., Beitia, F., Zchori-Fein, E., Vavre, F., Mouton, L., Moya, A., Latorre, A. and Silva, F.J. (2012) 'Complete genome sequence of "Candidatus *Portiera aleyrodidarum*" BT-QVLC, an obligate symbiont that supplies amino acids and carotenoids to *Bemisia tabaci*', *Journal of Bacteriology*, 194(23), pp. 6654-6655.

Saridaki, A. and Bourtzis, K. (2010) '*Wolbachia*: more than just a bug in insects genitals', *Current Opinion in Microbiology*, 13(1), pp. 67-72.

Signorile, A.L., Wang, J., Lurz, P.W.W., Bertolino, S., Carbone, C. and Reuman, D.C. (2014) 'Do founder size, genetic diversity and structure influence rates of expansion of North American grey squirrels in Europe?', *Diversity and Distribution*, 20(8), pp. 918-930.

Skaljac, M., Zanic, K., Ban, S.G., Kontsedalov, S. and Ghanim, M. (2010) 'Co-infection and localization of secondary symbionts in two whitefly species', *BMC Microbiology*, 10(1), p. 142.

Skaljac, M., Zanic, K., Hrncic, S., Radonjic, S., Perovic, T. and Ghanim, M. (2013) 'Diversity and localization of bacterial symbionts in three whitefly species (Hemiptera: Aleyrodidae)

546 from the east coast of the Adriatic Sea', *Bulletin of Entomological Research* 103(1), pp. 48-
547 59.

548 Suarez, A.V. and Tsutsui, N.D. (2008) 'The evolutionary consequences of biological
549 invasions', *Molecular Ecology*, 17(1), pp. 351-60.

550 Thao, M.L. and Baumann, P. (2004) 'Evolutionary relationships of primary prokaryotic
551 endosymbionts of whiteflies and their hosts', *Applied and Environmental Microbiology*,
552 70(6), pp. 3401-3406.

553 Tsagkarakou, A., Navajas, M., Papaioannou-Souliotis, P. and Pasteur, N. (1998) 'Gene flow
554 among *Tetranychus urticae* (Acari : Tetranychidae) populations in Greece', *Molecular*
555 *Ecology*, 7(1), pp. 71-79.

556 Tsagkarakou, A., Tsigenopoulos, C.S., Gorman, K., Lagnel, J. and Bedford, I.D. (2007)
557 'Biotype status and genetic polymorphism of the whitefly *Bemisia tabaci* (Hemiptera:
558 Aleyrodidae) in Greece: mitochondrial DNA and microsatellites', *Bulletin of Entomological*
559 *Research*, 97(1), pp. 29-40.

560 Tsuchida, K., Kudo, K. and Ishiguro, N. (2014) 'Genetic structure of an introduced paper
561 wasp, *Polistes chinensis antennalis* (Hymenoptera, Vespidae) in New Zealand', *Molecular*
562 *Ecology*, 23(16), pp. 4018-4034.

563 Weir, B.S. and Cockerham, C.C. (1984) 'Estimating F-statistics for the analysis of
564 population-structure', *Evolution*, 38(6), pp. 1358-1370.

565 Werren, J.H., Baldo, L. and Clark, M.E. (2008) '*Wolbachia*: master manipulators of
566 invertebrate biology', *Nature Reviews Microbiology*, 6(10), p. 741.

567 Werren, J.H., Skinner, S.W. and Huger, A.M. (1986) 'Male-killing bacteria in a parasitic
568 wasp', *Science (Washington)*, 231(4741), pp. 990-992.

569 Zchori-Fein, E. and Brown, J.K. (2002) 'Diversity of prokaryotes associated with *Bemisia*
570 *tabaci* (Gennadius)(Hemiptera: Aleyrodidae)', *Annals of the Entomological Society of*
571 *America*, 95(6), pp. 711-718.

572

573 Table 1**.** Collection sites, population codes, dates of collection, host plants, and genetic diversity indices for the glasshouse whitefly *T.*
574 *vaporariorum* populations from the UK examined in this study. The following genetic diversity indices are indicated: average number of alleles
575 per locus (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (A_r), and estimator
576 of fixation index (F).

Locality	Code	Year	Host	Plant family	Latitude	Longitude	N_a	N_e	H_o	H_e	A_r	F
Billingham East /Teesside	BE14	2014	Tomato	Solanaceae	54.604285	-1.257358	2.444	1.934	0.539	0.476	2.44	-0.106
Dundee	DU1_14	2014	Eupatorium	Asteraceae	56.456253	-3.025183	3.556	2.438	0.506	0.558	3.56	0.120
Dundee	DU15	2015	Eupatorium	Asteraceae	56.456253	-3.025183	2.667	2.197	0.594	0.495	2.67	-0.176
East Riding of Yorkshire	ERYS15	2015	Cucumber	Cucurbitaceae	53.741930	-0.731197	2.444	1.972	0.678	0.455	2.44	-0.469
East Riding of Yorkshire	ERYS14	2014	Cucumber	Cucurbitaceae	53.750523	-0.732015	3.222	1.945	0.517	0.444	3.22	-0.138
East York	EYO14	2014	Cucumber	Cucurbitaceae	53.771412	-0.748213	3.111	2.243	0.583	0.507	3.11	-0.125
Essex	ES15	2015	Tomato	Solanaceae	51.933305	1.022727	2.667	2.063	0.672	0.487	2.67	-0.360
Essex	ES14	2014	Tomato	Solanaceae	51.933305	1.022727	3.444	2.312	0.456	0.537	3.44	0.176
Herefordshire	HE2_14	2014	Cape gooseberry	Solanaceae	52.162737	-2.996278	3.111	2.197	0.650	0.482	3.11	-0.325
Herefordshire	HE3_14	2014	Basil	Lamiaceae	52.162737	-2.996278	3.000	1.969	0.478	0.417	3.00	-0.120
Herefordshire	HE4_14	2014	Chili peppers	Solanaceae	52.162737	-2.996278	3.000	1.982	0.556	0.430	3.00	-0.270
Herefordshire	HE15	2015	Squash	Cucurbitaceae	52.162737	-2.996278	2.222	1.951	0.589	0.443	2.22	-0.306
Isle of Wight	IW14	2014	Unknown	-	50.657994	-1.227233	3.333	2.362	0.544	0.464	3.33	-0.147
Kent County	KE15	2015	Tomato	Solanaceae	51.283319	1.295062	2.667	1.930	0.539	0.438	2.67	-0.205
Lab colony	LC15	2015	Eggplant	Solanaceae	54.980320	-1.615713	2.000	1.718	0.450	0.366	1.78	-0.204
Norfolk	NO15	2015	Tomato	Solanaceae	52.560526	0.442994	2.667	2.130	0.678	0.496	2.67	-0.343
Norfolk	NO3_14	2014	Tomato	Solanaceae	52.560526	0.442994	3.444	2.458	0.672	0.541	3.44	-0.219
Orkney	Or14	2014	Pelargonium	Geraniaceae	59.052969	-3.293660	3.222	2.564	0.683	0.576	3.22	-0.162
Orkney	Or15	2015	Pelargonium	Geraniaceae	59.052969	-3.293660	2.889	2.037	0.533	0.476	2.89	-0.096
West Sussex	WS15	2015	Tomato	Solanaceae	50.832853	-0.027808	2.333	1.967	0.600	0.459	2.33	-0.285
Mean							2.872	2.118	0.575	0.477	2.86	-0.188

577

578

Table 2. Pairwise estimates of genetic distance (F_{st}**)** values between 20 *T. vaporariorum* populations over the nine microsatellite loci. Significant values (p<0.05) are in **bold**.

POP	HE2_14	HE3_14	HE4_14	HE15	Or14	Or15	DU14	DU15	EYO14	ERYS15	ERYS14	ES15	ES14	WS15	NO15	NO14	IW14	BE14	KE15
HE3_14	0.03																		
HE4_14	0.02	0.02																	
HE15	**0.05**	**0.05**	**0.06**																
Or14	**0.17**	**0.22**	**0.21**	**0.21**															
Or15	**0.24**	**0.29**	**0.26**	**0.29**	**0.17**														
DU14	**0.14**	**0.15**	**0.14**	**0.16**	**0.12**	**0.13**													
DU15	**0.17**	**0.19**	**0.16**	**0.17**	**0.21**	**0.23**	**0.09**												
EYO14	**0.19**	**0.21**	**0.23**	**0.21**	**0.13**	**0.29**	**0.19**	**0.22**											
ERYS15	**0.14**	**0.19**	**0.14**	**0.15**	**0.23**	**0.28**	**0.21**	**0.23**	**0.26**										
ERYS14	**0.25**	**0.31**	**0.31**	**0.25**	**0.26**	**0.32**	**0.25**	**0.31**	**0.26**	**0.24**									
ES15	**0.11**	**0.16**	**0.14**	**0.13**	**0.18**	**0.17**	**0.16**	**0.19**	**0.18**	**0.21**	**0.24**								
ES14	**0.11**	**0.14**	**0.11**	**0.18**	**0.11**	**0.12**	0.05	**0.14**	**0.19**	**0.15**	**0.21**	**0.05**							
WS15	**0.21**	**0.22**	**0.21**	**0.22**	**0.23**	**0.16**	**0.18**	**0.23**	**0.29**	**0.16**	**0.31**	**0.21**	**0.17**						
NO15	**0.17**	**0.21**	**0.21**	**0.22**	**0.19**	**0.11**	**0.13**	**0.24**	**0.25**	**0.21**	**0.25**	**0.12**	**0.11**	**0.11**					
NO3_14	**0.03**	**0.07**	**0.06**	**0.05**	**0.14**	**0.21**	**0.09**	**0.14**	**0.14**	**0.08**	**0.16**	**0.06**	**0.03**	**0.14**	**0.12**				
IW14	**0.11**	**0.17**	**0.14**	**0.15**	**0.21**	**0.16**	**0.14**	**0.24**	**0.25**	**0.14**	**0.17**	**0.12**	**0.07**	**0.15**	**0.11**	0.04			
BE14	**0.18**	**0.21**	**0.22**	**0.18**	**0.23**	**0.21**	**0.17**	**0.22**	**0.17**	**0.23**	**0.25**	**0.13**	**0.17**	**0.16**	**0.11**	**0.12**	**0.13**		
KE15	**0.12**	**0.17**	**0.15**	**0.13**	**0.21**	**0.26**	**0.19**	**0.23**	**0.24**	**0.11**	**0.19**	**0.14**	**0.09**	**0.18**	**0.19**	**0.05**	**0.09**	**0.22**	
LC15	**0.17**	**0.24**	**0.21**	**0.19**	**0.26**	**0.23**	**0.21**	**0.29**	**0.27**	**0.26**	**0.33**	**0.13**	**0.14**	**0.25**	**0.18**	**0.14**	**0.13**	**0.12**	**0.25**

Table 3. Number of male and female individuals of *T. vaporariorum* infected by each of the seven endosymbiotic bacteria tested using specific and general primers for whitefly symbiotic bacteria.

Locality	Codes	N* ♀+♂	*Portiera* ♀+♂		*Wolbachia* ♀+♂		*Hamiltonella* ♀ + ♂		*Arsenophonus* ♀ + ♂		*Rickettsia* ♀ + ♂		*Cardinium* ♀ +♂		*Fritschea* ♀+♂	
Herefordshire	HE2_14	20	10	9	-		-	-	9	-	-	-	-		-	-
Herefordshire	HE3_14	20	10	10	-		-	-	10	-	-	-	-		-	-
Herefordshire	HE4_14	20	10	10	-		-	-	10	-	-	-	-		-	-
Herefordshire	HE15	20	10	10	-		-	-	10	-	-	-	-		-	-
Orkney	Or14	20	10	10	-		-	-	10	-	-	-	-		-	-
Orkney	Or15	20	10	10	-		-	-	10	-	-	-	-		-	-
Dundee	DU1	20	10	10	-		-	-	10	-	-	-	-		-	-
Dundee	DU15	20	10	10	-		-	-	10	-	-	-	-		-	-
East York's	EYO14	20	10	9	-		-	-	9	-	-	-	-		-	-
East Riding of Yorkshire	ERYS15	20	10	9	-		-	-	9	-	-	-	-		-	-
East Riding of Yorkshire	ERYS14	20	10	10	-		-	-	10	-	-	-	-		-	-
Essex	ES15	20	10	9	-		-	-	9	-	-	-	-		-	-
Essex	ES14	20	10	10	-		-	-	10	-	-	-	-		-	-
West Sussex	WS15	20	10	9	-		-	-	9	-	-	-	-		-	-
Norfolk	NO15	20	10	10	-		-	-	10	-	-	-	-		-	-
Norfolk	NO3_14	20	10	9	-		-	-	9	-	-	-	-		-	-
Isle of Wight	IW14	20	10	10	-		-	-	10	-	-	-	-		-	-
Billingham East /Teesside	BE14	20	10	10	-		-	-	10	-	-	-	-		-	-
Kent County	KE15	20	10	9	-		-	-	10	-	-	-	-		-	-
Lab Colony	LC15	20	10	9	-		-	-	9	-	-	-	-		-	-

* N, Number of male and female individuals.

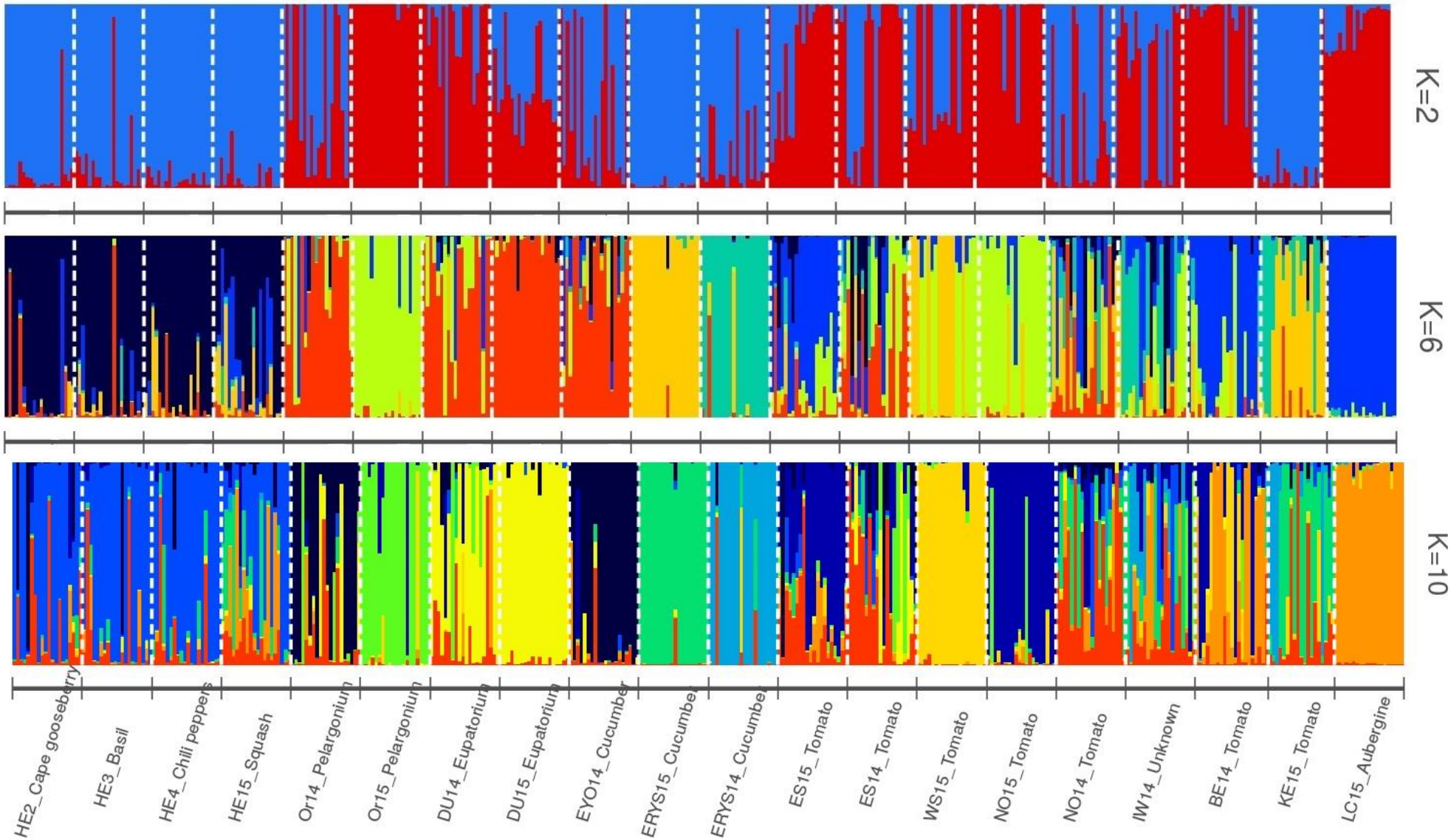

Figure 1**.** Genetic structure of 400 *T. vaporariorum* individuals (20 populations) based on nine microsatellite markers using the program STRUCTURE at K=2, 6 and 10. Each vertical bar represents the assignment of an individual. Colours indicate cluster assignment. Codes indicate location, year and host plant collections (See table 1).

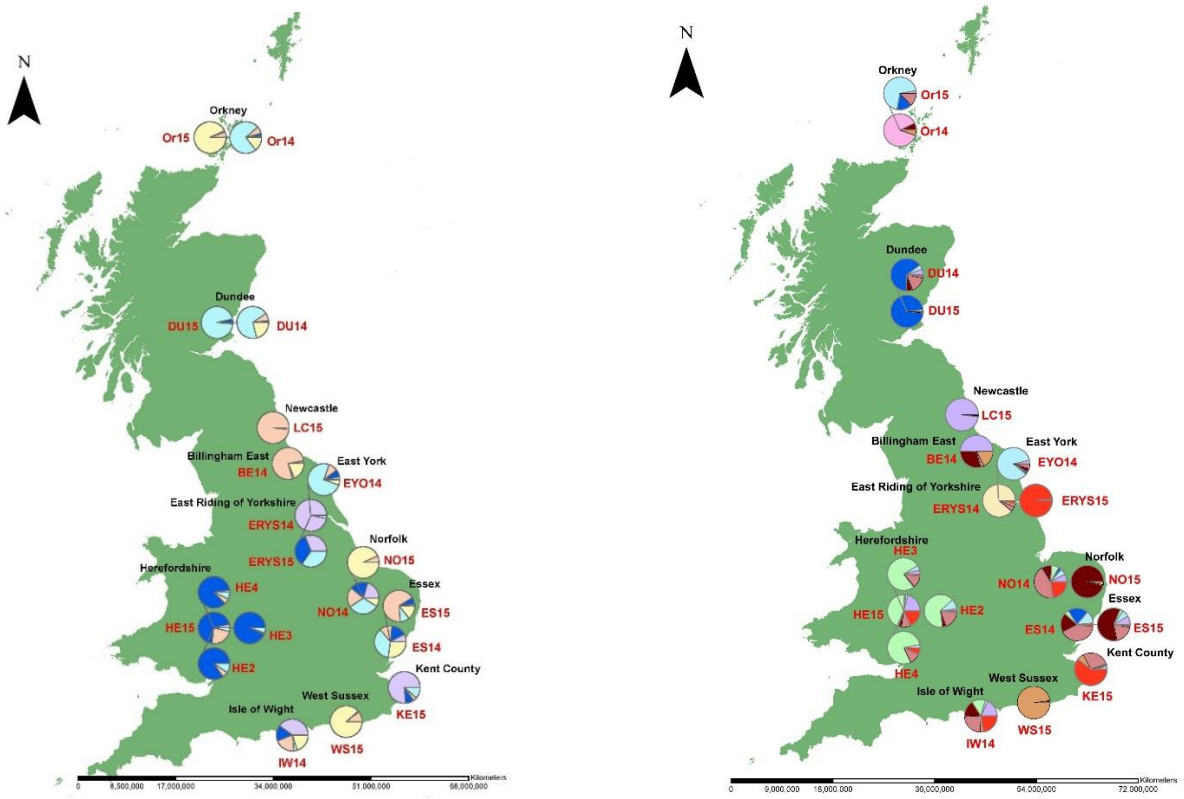

Figure 2. Geographical distribution of collection locations and the genetic structure of the *T. vaporariorum* revealed by STRUCTURE analysis with K: 6 (A) and K: 10 (B). Pie charts represent the proportion of Q values of each population (figure 1) and the codes of populations are listed in Table 1.