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

Ramiro Morales-Hojas[1*], Jingxuan Sun[2], Fernando Alvira Iraizoz[1], Xiaoling Tan[2], Julian Chen[2*]

[1] Rothamsted Insect Survey, Biointeractions and Crop Protection Department, Rothamsted Research, West Common, Harpenden AL5 2JQ, UK

[2] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2 West Yuanmingyuan Rd., Haidian District, Beijing, 100193, P. R. China

* Correspondence: Ramiro Morales-Hojas, e-mail: ramiro.morales-hojas@rothamsted.ac.uk, r.moraleshojas@gmail.com; Julian Chen, e-mail chenjulian@caas.cn

Running title: Population genetics of *Sitobion* in England and China

Abstract

Genetic diversity of populations has important ecological and evolutionary consequences, which are fundamental to improve the sustainability of agricultural production. Studies of how differences in agricultural management and environment influence the population structure of insect pests are fundamental to predict outbreaks and optimise control programmes. Here, we have studied the population genetic diversity and evolution of *Sitobion avenae* and *Sitobion miscanthi* (previously mistaken for *S. avenae*), which are among the most relevant aphid pests of cereals across Europe and China, respectively. We have used a genomic approach that allows the identification of weak geographic structure and migration patterns at scales that were previously not discernible. In the present study, we show that the population structure in present day populations are different from that described in previous studies, which suggests that they have evolved recently possibly as a response to human-induced changes in agriculture. In the UK, *S. avenae* is predominantly anholocyclic and, as a result of the evolution of insecticide resistance, a superclone is now dominant across the geographic distribution in the country and the genetic diversity is low. In China, *S. miscanthi* populations are mostly holocyclic, with one sexual stage in autumn to produce overwintering eggs, and there are six genetically differentiated subpopulations and high genetic differentiation between geographic locations, which suggests that further taxonomical research is needed. Unlike in the case of *S. avenae* in England, there is no evidence for insecticide resistance and there is no predominance of a single lineage in *S. miscanthi* in China.

Keywords: cereal aphids, population genetics, China, England, insecticide resistance

Introduction

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

Aphids comprise some of the most pernicious species of crop pests. In cereals, *Sitobion avenae* and *Sitobion miscanthi* are two of the most economically important species in Europe and Asia, respectively, and they are major vectors of the barley yellow dwarf virus (BYDV), which can severely reduce cereal yield (Vickerman & Wratten, 1979). Both *Sitobion* species are monoecious, feeding only on Poaceae grasses and cereals. Like many aphids, *S. avenae* and *S. miscanthi* show different levels of variation in the life-cycle, from individuals that are obligate cyclical parthenogenetic and

have a generation that undergoes sexual reproduction in the primary host (holocycly), to clones that are obligate parthenogenetic and reproduce asexually all year round (anholocycly) (Dedryver, Le Gallic, Gauthier, & Simon, 1998). In addition, individuals can remain asexual in the cereal crops during winter as a response to environmental cues such as warmer temperatures and day length. As a result, a geographic cline in the reproductive type has been described in the *S. avenae* populations of UK and France, with increasing proportion of sexual reproduction towards the north of the countries (Llewellyn et al., 2003; Simon et al., 1999). In the case of *S. miscanthi*, variation in the life-cycle has also been described. Populations from this species in Australia and New Zealand are anholocyclic, while they are holocyclic in Taiwan (Sunnucks, England, Taylor, & Hales, 1996; Wilson, Sunnucks, & Hales, 1999). In China, *S. miscanthi* has been traditionally reported to be anholocyclic (Guo, Shen, Li, & Gao, 2005; Zhang, 1999). However, contrary to the observations in *S. avenae* and other species, a recent population genetics study has observed signatures of cyclical parthenogenesis in the southern populations of the country while obligate parthenogenetic reproduction would be dominant in the north (Wang, Hereward, & Zhang, 2016).

Resistance to pyrethroids was first detected in the UK populations of *S. avenae* in 2011. This was due to a knockdown resistance (*kdr*) mutation (L1014F) in the sodium channel gene (Foster et al., 2014). This mutation appeared in heterozygosity in one clone of *S. avenae*, known in the literature as clone SA3, and rapidly increased its abundance in the UK population from 2009 to 2014, although in variable proportions in different locations and years (Dewar & Foster, 2017; Malloch, Foster, & Williamson, 2016; Malloch, Williamson, Foster, & Fenton, 2014). The spread of the mutation in the UK was limited by the fact that the SA3 clone is anholocyclic, so pyrethroid resistance has not spread to other lineages through sexual recombination. In addition, the high connectivity of the UK populations (Llewellyn et al., 2003), probably facilitated the geographic spread of the resistant clone from its location of origin. Therefore, the continued use of pyrethroids combined with the long dispersal capacity of *S. avenae* has likely favoured the spread of this clone across the UK.

Nevertheless, the clonal diversity remained high and similar to the diversity before the evolution of

pyrethroid resistance, and other susceptible clones and phenotypes were still present in different proportions in British populations by 2015 (Llewellyn et al., 2003; Malloch et al., 2016). In the case of *S. miscanthi*, there is no available information in the literature regarding the evolution of insecticide resistance. However, understanding the dynamics and movement of the species can help manage and control the damage in cereal crops, and establish management programs to reduce the likelihood of insecticide resistance evolution. In China, previous studies have shown high levels of genetic diversity in *S. miscanthi* (Guo et al., 2005; Wang et al., 2016), similar to those reported for *S. avenae* in the UK and France, and there is genetic differentiation between north and south of the country but low differentiation within each region, suggesting free gene flow within geographic regions (Guo et al., 2005; Wang et al., 2016).

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

Materials and Methods

Samples

Individuals of *S. avenae* were collected during June-July 2018 using the 12 English 12.2 m high suction-traps ran by Rothamsted Insect Survey (Table 1). Individuals of *S. miscanthi* were collected in 10 sites across the cereal growing areas of China (Table 1). The 10 individuals of *S. miscanthi* were collected from the same wheat field but from plants separated by 10 m to reduce the probability of sampling the same clone.

DNA extraction and SNP genotyping

DNA was extracted from samples using Qiagen's DNeasy Blood and Tissue kit following the manufacturer's protocol. Samples of *S. avenae* and *S. miscanthi* were genotyped separately. Genomic DNA was digested with *MseI* in the case of *S. avenae* and with *ApeKI* in the case of *S. miscanthi*. The library preparation was performed following the standard Illumina pair-end (PE) protocol, and PE sequencing of 150 bp was performed on an Illumina HiSeq platform. Library preparation and sequencing of samples were outsourced commercially. Reads quality was assessed with FastQC v0.67 and, in the case of *S. miscanthi*, the first 10 bases were trimmed due to low quality using trimmomatic 0.36.1 (Bolger, Lohse, & Usadel, 2014). Reads were mapped to a draft of the *S. avenae* genome. Duplicates were removed using MarkDuplicates v2.7.1.1 and indels were realigned with BamLeftAlign v1.0.2.29-1. Variant calling was carried out with FreeBayes v1.0.2.29-3 (Garrison & Marth, 2012). The resulting SNPs from FreeBayes were annotated using snpEff v4.0. These tools were run using Galaxy v17.05 (Afgan et al., 2016). SNPs called with FreeBayes were filtered using VCFtools v0.1.14 (Danecek et al., 2011) before the markers were used in subsequent analyses. Different filtering schemes were used to obtain a dataset that maximised the quality of the SNPs and genotypes while minimising the missing data at marker and individual levels (Supplementary Table S1), as recommended by O'Leary, Puritz, Willis, Hollenbeck, and Portnoy (2018).

Analyses of population structure

The population structure of both species was investigated using the Bayesian genetic clustering algorithm implemented in Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). We used the admixture model with correlated frequencies and, to detect any potential subtle genetic structure, we ran Structure with the sampling locations set as priors (locprior = 1); this model has the power to detect a weak structure signal and does not bias the results towards detecting genetic structure when there is none. In the case of *S. miscanthi*, a first run of Bayesian clustering analyses of the population structure was carried out with 5 independent simulations with 100,000 burn-in and

100,000 mcmc chains for each of *K* 1–10. An additional run of 5 independent simulations with 100,000 burn-in and 500,000 mcmc chains was carried out for *K* 5–10 to confirm the results of the first run and ensure convergence in the mcmc step. In the analyses of *S. avenae* from the UK, Structure was run with 5 replicates of 500,000 burn-in and 1,000,000 mcmc chains for *K* ranging from 1 to 12. Parameter convergence was inspected visually. We ran the Structure simulations using a multi-core computer with the R package ParallelStructure (Besnier & Glover, 2013) in the CIPRES science gateway server (Miller, Pfeiffer, & Schwartz, 2010). The number of *K* groups that best fitted the dataset was estimated using the method of Evanno, Regnaut, and Goudet (2005) using Structure Harvester Web v0.6.94 (Earl & Vonholdt, 2012). Cluster assignment probabilities were estimated using the programme Clumpp (Jakobsson & Rosenberg, 2007) as implemented in the webserver CLUMPAK (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015).

The genetic diversity measures of the populations were estimated using Arlequin 3.5.2.2 (Excoffier, Laval, & Schneider, 2005). Genetic variation among populations was investigated using an Analysis of the Molecular Variance (AMOVA) with 10000 permutations using Arlequin 3.5.2.2. We used hierarchical AMOVA to test the population structures resulting from the Structure runs. Population pairwise divergence was investigated using F_{ST}, and the significance was evaluated with 10000 permutations in Arlequin. We ran a Mantel test as performed in Arlequin to evaluate the correlation between the genetic distances (F_{ST}) and the geographic distance between sampling locations in China estimated using Google maps (Supplementary Table S2). The demographic history of *S. avenae* was also explored using Arlequin. For this, we first estimated the gametic phase from the multilocus diploid data using the ELB algorithm with the default parameter values. Population expansion or bottleneck were inferred using Fu's F_S (Fu, 1997), and mismatch analyses were run with 1000 bootstrap replicates to estimate the Harpending's raggedness index and the sum of squared deviation (SSD) given the population expansion and spatial expansion models.

Phylogenetic trees for *S. avenae* were constructed using Maximum Likelihood (ML) with RAxML 8.2.12 (Stamatakis, 2014) run in the server CIPRES (Miller et al., 2010). RAxML was run with 1000

bootstrap inferences with subsequent ML search using the gtrgamma model. The Lewis correction for ascertainment bias was implemented as it is the appropriate model for binary datasets that include only variable sites (as it is the case of SNPs) (Leache, Banbury, Felsenstein, de Oca, & Stamatakis, 2015; Lewis, 2001).

Results

Genetic diversity and population structure of S. miscanthi *in China*

A total of 14520 SNPs (with less than 20% of missing data per individual and 10% per locus, Supplementary Figures S1) were obtained for the 100 individuals from the 10 Chinese populations. The levels of gene diversity (He) observed across all populations are lower than in previous studies of *S. miscanthi* in China, but similar to that of the UK population of *S. avenae* and other cereal aphids like *R. padi* (Morales-Hojas et al., 2019) (Table 2). Overall, the Chinese population is not in Hardy Weinberg Equilibrium (HWE), and the inbreeding coefficient is positive (Table 2). The same positive, significant F_{IS} is observed when we group the populations into North China (Qingdao, Tai'an, Langfang, Taigu, Pingliang and Yinchuan) and South China (Shuzou, Wuhan, Mianyang and Kunming) following the Qingling-Huaihe line (QHL; the traditional identified geographic North-South divide of China) (Table 2). Furthermore, several populations in China (Qingdao, Taigu, Yinchuan and Wuhan) of are also not in HWE, with significant, positive F_{IS} indices. Significant, positive estimates of F_{IS} is generally the result of inbreeding in the population or the effect of population subdivision, the Wahlund effect.

A first run of Bayesian clustering analyses of the population structure was carried out with 5 independent simulations with 100,000 burn-in and 100,000 mcmc chains for each of K 1–10. Analyses of the results following the Evanno method (Evanno et al., 2005) indicated that the most likely number of clusters was K = 6. An additional run of 5 independent simulations with 100,000 burn-in and 500,000 mcmc chains carried out for K 5–10 confirmed that the most likely number of K was 6 (Table 3, Figure 1). The structure plot shows that most sampled locations are not

190 homogeneous, comprising individuals that are assigned to different genetic clusters (Figure 1). These
191 results suggest that the Wahlund effect, admixture of different populations, is the most likely reason
192 for the significant F_{IS} in Qingdao, Taigu, Yinchuan and Wuhan, which are the populations with more
193 admixture. This is further supported by the fact that F_{IS} is non-significant when the individuals are
194 grouped according to the genetic cluster to which they were assigned by the Bayesian clustering
195 analyses with Structure and thus, genetic clusters are in HWE except GC2 that includes 27 individuals
196 from seven different locations (Table 2).
197 Analysis of the molecular variance (AMOVA) indicated that the overall F_{ST} of *S. miscanthi* in China
198 was 0.3254 (P = 0); thus, 32.54% of the total genetic variation was explained by differences between
199 the populations. When the individuals were grouped according to their assigned genetic cluster,
200 40.68% of the genetic variation (F_{CT} = 0.4069, P = 0) was explained by differences among the groups,
201 indicating that the grouping of individuals from different populations but with the same genetic
202 background increased the proportion of genetic variation explained (Table 4). Finally, we tested the
203 QHL North-South subdivision of the *S. miscanthi* population previously suggested in the literature
204 with an AMOVA. Results did not support the QHL divide hypothesis with only a non-significant 2.22%
205 of the genetic variation being explained by such geographic division (F_{CT} = 0.0222, P = 0.2569) (Table
206 4).
207 Pairwise F_{ST} tests showed that the genetic differentiation between the different populations is high
208 and significant in most cases (Table 5). The only exceptions are between the populations of Suzhou
209 and Pingliang, and Yinchuan and Taigu, which showed no genetic differentiation. Qingdao and
210 Tai'an, which are separated by 300 kms, showed a low but significant F_{ST}, and Langfang, Pingliang,
211 Mianyang and Suzhou showed an intermediate and significant F_{ST}, despite some of these locations
212 being more than 1000 kms apart. The genetic differentiation between the genetic clusters is higher
213 and significant in all pairwise comparisons (Table 6).
214 The Mantel test was non-significant when no genetic structure within *S. miscanthi* is considered,
215 rejecting the isolation by distance (IBD) hypothesis. However, as the Bayesian clustering analyses

showed, there are 6 likely genetic clusters and this subdivision of the population can bias the test. To control for this, we performed Mantel tests for each of the identified clusters 2, 5 and 6 separately (these clusters included individuals from more than one location) but results were still non-significant. Nevertheless, the low number of individuals for some of the locations within each cluster limits the statistical power of the tests.

Genetic diversity and population structure of S. avenae *in the UK*

A total of 846 SNPs with less than 25% of missing data per locus and <50% per individual (Supplementary Figure S2) were identified in 98 individuals from 12 sampling locations across England. The gene diversity, estimated as the He, is high across all populations (Table 7), although it is lower than the He observed in a previous analysis of the UK populations using microsatellites (Llewellyn et al., 2003). The Ho was higher in all populations compared to the He, resulting in high negative F_{IS} estimates which indicate that the *S. avenae* in England is not in HWE (Table 7). This contrasts with the situation of the UK population 15-20 years ago where most markers in the analysed populations were in HWE (Llewellyn et al., 2003). Although the negative F_{IS} values were not significant according to the permutation test performed by Arlequin 3.5, it should be noted that this test evaluates the probability of obtaining random values that are higher than the observed ones rather than the probability of random values being more negative. Therefore, it is most probable that the P values reported are not correct for these values. The negative F_{IS} is the result of an excess of heterozygotes, and it is considered a signature of clonal reproduction.

Bayesian clustering analysis was run with 5 replicates of 500,000 burn-in and 1,000,000 mcmc chains to ensure convergence. Results were analysed following the Evanno method (Evanno et al., 2005), which suggested that the most likely number of genetic clusters is K = 2 (Table 8). However, this method does not estimate the deltaK for K = 1 and the Mean LnP(K) is maximised for K = 1 suggesting that the most likely number of clusters is 1 (Table 8). In addition, the standard deviation increases from K = 2, which usually happens after reaching the best K. Finally, there is no clear

242 distribution of individuals into 2 differentiated clusters in the bar plot resulting from the analyses
243 with k = 2, with all of them having some probability of belonging to each cluster (Figure 2b). These
244 results suggest that there is no population structure but just one genetic cluster.
245 Analyses of the Molecular Variance (AMOVA) also supported the existence of one single gene
246 cluster. The overall diversity of *S. avenae* in England was low F_{ST} = 0.001 and non-significant among
247 populations, indicating a low differentiation level. When the individuals were clustered according to
248 the Structure results for K = 2, assigning individuals to each genetic cluster according to the
249 membership coefficient estimated with clump, the amount of genetic variation that was explained
250 between groups was 2.33% (P = 0) (Table 9). These results support those of Structure with one single
251 genetic cluster and no population structure. In addition, the genetic differentiation estimates (F_{ST})
252 were all negative (Table 10), which indicates that there is no divergence between the different
253 locations. Similarly, when the two possible genetic clusters identified with Structure are compared,
254 the F_{ST} is low (0.0158; P = 0), further supporting the lack of population structure in *S. avenae* from
255 England.
256 Phylogenetic analyses were run using the two multi-SNP haplotypes of each individual. The inferred
257 ML phylogeny comprised two major clades highly supported by bootstrap (Figure 3). Each of these
258 two clades included one of the multi-SNP haplotypes from each individual, so that each multi-SNP
259 haplotype is more closely related to a haplotype from another individual than to the second
260 haplotype from the same individual. This type of phylogenetic topology can be the result of asexual
261 reproduction from a single individual, in which all copies from each of the extant haplotypes derive
262 from one common ancestral haplotype with no recombination. The basal node would represent the
263 common ancestral clonal individual. Thus, the phylogeny supports the lack of population structure,
264 and suggests that there is one single clone dominating the English population of *S. avenae*, and the
265 asexual reproduction of this lineage.
266 Analyses of the historical demography of this species in England showed a population and spatial
267 expansion, which would be consistent with the increase in frequency of a single insecticide-resistant

clone in the population and its spread across different locations. Thus, Fu's F_s index was negative (F_s = -25.41, P = 0), which is a signature of population expansions. In addition, the mismatch analyses showed a unimodal distribution and failed to reject departure from the expansion models, resulting in a non-significant Harpending's raggedness index (0.0001, P = 1) for the demographic and spatial expansion models, and non-significant sum of squared deviation (SSD) for the spatial expansion model (P = 0.438) while significant at the 5% for the population expansion model (P = 0.031).

Discussion

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

Results of the study indicate that *S. miscanthi* in China has a higher diversity than previously identified (Guo et al., 2005; Wang et al., 2016). This can be explained by the higher number of genome-wide molecular markers that have been used in the present analysis, but the estimated levels of genetic differentiation were similar to those of the *S. avenae* population in China (Xin, Shang, Desneux, & Gao, 2014). The species *S. avenae* is known to be present only in Yili, Xinjiang region in the northwest (Zhang, 1999), so it is likely that some previous studies of this species in China have used the incorrect taxonomic name. In addition, it could also be the case that there is undescribed taxonomic diversity within *S. miscanthi* in China, and the high levels of genetic differentiation observed could be explained by unidentified races. This is the case in Australia, where there are at least three chromosomal races (Hales, Chapman, Lardner, Cowen, & Turak, 1990), so it

294 is possible that the situation in China is complex as well. Indeed, the high genetic differentiation
295 observed in the present analysis between the six genetic clusters identified suggests that there are
296 at least the same number of different taxonomic units, though the present study is not capable to
297 determine whether they represent subspecies, host races or chromosomal races as in Australia and
298 New Zealand.
299 While *S. miscanthi* in Australia and New Zealand is predominantly functional parthenogenetic
300 (Sunnucks et al., 1996; Wilson et al., 1999), the present study indicates that the populations in China
301 show either a heterozygote deficiency (significant, positive F_{IS}) or are in HWE (not significant F_{IS}),
302 suggesting that the species reproduces predominantly by cyclical parthenogenesis. The significant
303 deficiency of heterozygosity in populations can be explained by the admixture of different genetic
304 clusters (Wahlund effect) observed in several populations, and the F_{IS} is not significant for the
305 genetic clusters indicating that they are in HWE (with the exception of the GC2). This contrasts with
306 previous studies that inferred an excess of heterozygotes in the northern populations, characteristic
307 of anholocyclic lineages, while in the southern populations there was a deficiency of heterozygosity,
308 which is observed in inbred sexual populations or the result of admixed populations (Wahlund
309 effect) (Wang et al., 2016). However, it is usually the case that in colder regions populations are
310 cyclical parthenogenetic as the aphids undergo a phase of sexual reproduction to produce eggs to
311 overwinter; on the other hand, southern populations in warmer regimes can survive as
312 parthenogenetic individuals throughout the year. The results of Wang et al. (2016) indicate that the
313 contrary would be the case in Chinese *S. miscanthi*, which is unexpected. Other study of *S. miscanthi*
314 in China showed that northern populations had heterozygote deficiency while the southern
315 populations were in HWE or had an excess of heterozygotes, although the significance was not
316 tested (Guo et al., 2005). The contrasting results between the present and previous analyses are also
317 at the population subdivision. Thus, we observe no significant north-south differentiation at the QHL
318 traditional division, identified in one previous study (Wang et al., 2016), and there is no evidence for
319 isolation by distance (Guo et al., 2005). The population structure identified in the present study

suggests that there are six genetic clusters highly differentiated. These clusters do not correspond to geographic regions and individuals from populations geographically separated by long distances can belong to the same genetic cluster; only GC1 comprising individuals from only Kunming and GC3 with individuals only from Wuhan are geographically restricted to one location. This suggests that there is long distance dispersal of *S. miscanthi* aphids across China, although the high differentiation observed between the genetic clusters suggest low interbreeding between them.

In England, the level of genetic differentiation is low across the different populations of *S. avenae* sampled and there is no evidence for genetic structure. This is in accordance to what it was previously observed (Llewellyn et al., 2003). This population homogeneity was taken to be the result of long distance dispersal of aphids, and results from the present study corroborates this. The population of *S. avenae* in England, however, has evolved in the last 15 years. While most of the markers and populations studied in 1997-1998 were in HWE, and the population showed an increase in cyclical parthenogenetic proportion with latitude (Llewellyn et al., 2003), this study shows that the present English population has an excess of heterozygotes and indicates strong clonality. This suggests that anholocycly is predominant across its range, and there is no evidence for cyclical parthenogenesis occurring towards the north of the country as expected. This change in the *S. avenae* population is most likely the result of insecticide resistance evolution. In 2011, a knockdown resistance (*kdr*) mutation to pyrethroids was detected in England's population of *S. avenae*, and studies showed that the clone that gained this mutation spread and increased its proportion in the population from 2009 to 2014, and was also observed in Ireland from 2013 (Dewar & Foster, 2017; Foster et al., 2014; Malloch et al., 2016; Malloch et al., 2014; Walsh et al., 2019). It is therefore likely that this pyrethroid-resistant clone, which is a facultative parthenogenetic clone (Walsh et al., 2019), has continued to spread and increase in proportion, being now dominant in the English population. This is supported by the phylogenetic analysis, which shows a topology characteristic of clonal organisms. Also, the levels of genetic diversity (as measured by the He) are now lower than those of 2003 (Llewellyn et al., 2003). Although the markers used are different, it would be expected that

346 using more, genome-wide SNPs would provide a higher level of diversity than a limited number of
347 microsatellites. In addition, analyses of the demographic history of the population in England
348 indicates that there has been a population demographic and spatial expansion, which substantiates
349 the increase in proportion of the insecticide-resistant clone. Thus, as one clone gained the resistance
350 to pyrethroids in a given location, it increased in number in the location but also expanded its
351 distribution as it spread to other regions via migration.
352 Overall, this study demonstrates that the populations of two close species of cereal aphids of the
353 genus *Sitobion* have evolved in recent years in two geographically distant regions under different
354 environmental and human-influenced conditions. The diversity of *S. miscanthi* in China needs to be
355 investigated more comprehensively, as the high level of genetic differentiation suggests the
356 existence of yet unidentified forms. In contrast, the diversity of *S. avenae* has been affected by the
357 evolution of pyrethroid resistance, and a single clone appears to be now dominating the English
358 population. In contrast to this, *S. miscanthi* has not gained insecticide resistance despite having been
359 subject also to its use. In England, the bird cherry – oat aphid, *R. padi*, has not evolved resistance to
360 insecticides either, despite being sympatric with *S. avenae* and therefore subject to the same
361 agricultural practices. Why some species evolve resistance while other do not it is still a matter of
362 study. It has been shown in *Drosophila melanogaster* that thermotolerance influences the
363 development and spread of insecticide resistance (Fournier-Level et al., 2019). Similarly, the
364 distribution of cyclical and obligate parthenogenetic aphids is strongly influenced by temperature, so
365 it is possible that there is a relationship between life-cycle type and insecticide resistance evolution
366 in aphids. This would explain the evolution of the *kdr* and super-*kdr* mutations in the population of *S.*
367 *avenae* in England, where it is predominantly anholocyclic, while the sympatric population *R. padi*
368 and the closely related species *S. miscanthi* in China, which are predominantly cyclical
369 parthenogenetic, have not.
370

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Data Accessibility

All the DNA sequencing reads have been uploaded to the European Nucleotide Archive (ENA) and can be found under the study with accession number PRJEB36151. Accession numbers for the samples' sequencing reads are ERR3810098 – ERR3810316.

Authors Contributions

RMH designed the project, performed the research, analysed the data, wrote and revised the manuscript.

JS collected samples, performed the research, revised the manuscript.

FAI performed the research, revised the manuscript.

XT performed the research, revised the manuscript.

JC designed the project, revised the manuscript.

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

Country	Location	Geographic coordinates	N
UK	Broom's Barn (BB)	52.260681, 0.56843	10
UK	Hereford (H)	52.124201, -2.638156	10
UK	Kirton (K)	52.924454, -0.052153	10
UK	Newcastle (N)	55.213254, -1.685083	10
UK	Preston (P)	53.854383, -2.76699	10
UK	Rothamsted (RT)	51.806997, -0.360091	10
UK	Silwood Park (SP)	51.40941, -0.643357	10
UK	Starcross (SX)	50.629596, -3.45463	10
UK	Wellesbourne (We)	52.205975, -1.605017	10
UK	Writtle (Wr)	51.733599, 0.429233	10
UK	Wye (W)	51.185507, 0.944941	10
UK	York (Y)	54.014616, -0.97320532	10
China	Kunming (KM)	24.8855, 102.8215	10
China	Mianyang (MY)	31.5347, 104.5676	10
China	Wuhan (WH)	30.5820, 114.0292	10
China	Qingdao (QD)	36.3074, 120.3963	10
China	Tai'an (TA)	36.1920, 117.1353	10
China	Pingliang (PL)	35.5426, 106.6748	10
China	Yinchuan (YC)	38.4731, 106.2428	10
China	Langfang (LF)	39.5031, 116.6857	10
China	Taigu (TG)	37.4212, 112.5513	10
China	Suzhou (SZ)	31.3023, 120.6313	10

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516
517 **Table 2.** Mean genetic diversity indices estimated for each *S. miscanthi* population, populations
518 north and south of the QHL and each of the identified genetic clusters (GC). Ho and He are observed
519 and expected (gene diversity) heterozygosity, respectively; F_{IS} – inbreeding coefficient.

	He	Ho	F_{IS}	*P(random F_{IS} ≥ observed F_{IS})*
Overall	0.28933	0.16720	0.3871	0.0000
North	0.33122	0.19451	0.37625	0.0000
Qingdao	0.3796	0.30129	0.16357	*0.0117*
Tai'an	0.46466	0.44985	-0.01467	0.5147
Langfang	0.42943	0.40584	0.00621	0.4766
Taigu	0.2948	0.22396	0.21015	0.0407
Pingliang	0.41717	0.34735	0.08827	0.1097
Yinchuan	0.30452	0.22792	0.24868	0.0344
South	0.30482	0.19169	0.33871	0.0000
Shuzou	0.36834	0.313	0.10903	0.0689
Wuhan	0.3134	0.19447	0.36651	0.0004
Mianyang	0.46364	0.4364	-0.00632	0.4827
Kunming	0.35638	0.33113	0.05435	0.2473
GC1	0.46035	0.49644	-0.10550	0.8083
GC2	0.40616	0.35361	0.07863	0.0447
GC3	0.45541	0.42329	-0.01105	0.5731
GC4	0.41227	0.37039	0.05263	0.1626
GC5	0.36980	0.38917	-0.08655	0.8816
GC6	0.42755	0.43997	-0.05740	0.5807

520 **Table 3.** Table of results from Structure for the Chinese populations (5 independent simulations for K
521 5 – 10, 100,000 burn-in and 500,000 mcmc chains).

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
5	5	-962395	53822.58	NA	NA	NA
6	5	-949885	94126.26	12510.06	49025850	520.852
7	5	-5E+07	49016699	-4.9E+07	55801634	1.138421
8	5	-4.3E+07	59145788	6788294	28389784	0.479997
9	5	-6.5E+07	41036977	-2.2E+07	59738205	1.455717
10	5	-2.7E+07	23812471	38136715	NA	NA

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Table 4. AMOVA of the SNP dataset from *S. miscanthi*. Analyses were performed to test the following hierarchical substructure: (A) individuals grouped according to the genetic cluster assignment from Structure; (B) populations grouped according to their North-South location with respect to the Qingling-Huaihe Line divide.

A	Source of variation	Sum of squares	Variance components	% variation	Fixation Indices
	Among groups	146960.625	840.81437	40.68	F_{CT} = 0.4068 (P = 0)
	Among populations within groups	23445.277	83.52677	4.04	F_{SC} = 0.0681 (P = 0)
	Among individuals within populations	90706.443	-22.79868	-1.10	F_{IS} = -0.0199 (P = 0.6556)
	Within individuals	116543.000	1165.43000	56.38	F_{IT} = 0.4362 (P = 0)
B	Among groups	17982.662	43.99279	2.22	F_{CT} = 0.0222 (P = 0.2569)
	Among populations within groups	110074.833	614.04835	31.01	F_{SC} = 0.3172 (P = 0)
	Among individuals within populations	133054.850	156.47861	7.90	F_{IS} = -0.1184 (P = 0)
	Within individuals	116543.000	1165.43000	58.86	F_{IT} = 0.4114 (P = 0)

Table 5. Genetic differentiation between populations estimated with pairwise F_{ST} (below the diagonal) and significance (P values, above the diagonal) of the exact test estimated with 10100 permutations.

	South populations				North populations					
	Kunming	**Mianyang**	**Wuhan**	**Suzhou**	**Yinchuan**	**Pingliang**	**Taigu**	**Tai'an**	**Langfang**	**Qingdao**
Kunming		0	0.0001	0.0001	0	0	0	0	0.0001	0
Mianyang	0.38556		0.0003	0	0	0.0001	0	0	0.0001	0
Wuhan	0.35288	0.25497		0.0002	0	0.0001	0.0001	0.0003	0.0002	0.0009
Suzhou	0.35113	0.13993	0.20726		0.0004	0.9374	0	0	0	0.0003
Yinchuan	0.47602	0.36352	0.48536	0.31532		0.0012	0.9553	0	0	0
Pingliang	0.36378	0.12852	0.22950	-0.00006	0.31710		0.0003	0	0	0
Taigu	0.52138	0.43957	0.54790	0.39557	-0.01011	0.40377		0.0001	0	0
Tai'an	0.40604	0.37101	0.23802	0.29632	0.46615	0.33196	0.52301		0	0.005
Langfang	0.37918	0.13704	0.26178	0.11658	0.34895	0.12602	0.42251	0.35654		0.0001
Qingdao	0.34611	0.27895	0.17379	0.20910	0.40618	0.24716	0.46340	0.05756	0.27520	

Table 6. Genetic differentiation (pairwise F_{ST}) between the six genetic clusters (GC) identified with Structure. All pairwise comparisons were significant (P = 0) as estimated with 10100 permutations. GC 1 includes only individuals (n = 9) from Kunming; GC 2 comprises individuals from Kunming (1), Langfang (10), Mianyang (10), Pingliang (2), Qingdao (1), Suzhou (1) and Wuhan (2); GC 3 consists of individuals from Wuhan (6); GC 4 includes individuals collected in Taigu (9) and Yinchuan (8); GC 5 is formed by individuals from Qingdao (9), Suzhou (1), Tai'an (10) and Wuhan (2); and GC 6 includes individuals from Pingliang (8), Suzhou (8), Taigu (1) and Yinchuan (2).

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

Table 7. Mean genetic diversity indices estimated for each *S. avenae* population in England and overall. N is the number of gene copies (2 x number of individuals); Ho and He are observed and expected (gene diversity) heterozygosity, respectively; F_{IS} – inbreeding coefficient. Starcross values are not included as it was represented by one single individual.

	N	He	Ho	F_{IS}	$P(\text{random } F_{IS} \geq \text{observed } F_{IS})$
Overall	196	0.28433	0.41243	-0.6928	1
Broom's Barn	20	0.37289	0.54525	-0.6631	1
Hereford	20	0.37214	0.53194	-0.8069	1
Kirton	18	0.38078	0.54714	-0.7750	1
Newcastle	20	0.38088	0.56614	-0.7302	1
Preston	12	0.40722	0.57489	-0.7154	1
Rothamsted	16	0.37388	0.51535	-0.6035	1
Silwood Park	14	0.39285	0.54213	-0.7222	1
Starcross	2	-	-	-	-
Wye	18	0.36667	0.51283	-0.7137	1
Wellesbourne	20	0.35591	0.50472	-0.6003	1
Writtle	18	0.36290	0.51392	-0.6405	1
York	18	0.37310	0.53559	-0.7419	1

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

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	5	-55651.5	0.8927	NA	NA	NA
2	5	-59437.7	795.9415	-3786.2	4110.34	5.164123
3	5	-67334.3	1512.473	-7896.54	4447.6	2.940614
4	5	-70783.2	4688.426	-3448.94	3568.64	0.761159
5	5	-70663.5	3032.39	119.7	6005.16	1.980339
6	5	-76549	5716.411	-5885.46	12612.16	2.206308
7	5	-69822.3	2700.041	6726.7	9361.38	3.467125
8	5	-72456.9	7835.535	-2634.68	7552.04	0.963819
9	5	-67539.6	3157.942	4917.36	4039.52	1.279162
10	5	-66661.7	4041.049	877.84	2479.22	0.613509
11	5	-63304.7	2208.609	3357.06	5218.48	2.362791
12	5	-65166.1	3124.338	-1861.42	NA	NA

Table 9. AMOVA of the SNP dataset from *S. avenae*. Analyses were performed to test the genetic cluster assignment from Structure, K = 2.

A	Source of variation	Sum of squares	Variance components	% variation	Fixation Indices
	Among groups	203.571	1.87014	2.33	F_{CT} = 0.0233 (P = 0)
	Among populations within groups	397.908	-0.21891	-0.27	F_{SC} = -0.0028 (P = 0.8146)
	Among individuals within populations	1765.164	-55.77004	-69.41	F_{IS} = -0.7087 (P = 1)
	Within individuals	13177.500	134.46429	167.36	F_{IT} = -0.6736 (P = 1)

Table 10. Genetic differentiation between populations estimated with pairwise F_{ST} (below the diagonal). BB – Broon's Barn; H – Hereford; K – Kirton; N – Newcastle; P – Preston; RT – Rothamsted; SP – Silwood Park; SX – Starcross; W – Wye; We – Wellesbourne; Wr – Writtle; Y – York.

	BB	H	K	N	P	RT	SP	SX	W	We	Wr	Y
BB	0.0000											
H	-0.0195	0.0000										
K	-0.0311	-0.0297	0.0000									
N	-0.0051	-0.0244	-0.0438	0.0000								
P	-0.0206	-0.0454	-0.0590	-0.0499	0.0000							
RT	-0.0321	-0.0410	-0.0466	-0.0281	-0.0433	0.0000						
SP	-0.0292	-0.0471	-0.0419	-0.0374	-0.0528	-0.0549	0.0000					
SX	-0.4919	-0.4619	-0.4359	-0.5208	-0.5768	-0.5711	-0.4966	0.0000				
W	-0.0329	-0.0328	-0.0420	-0.0269	-0.0430	-0.0532	-0.0470	-0.4958	0.0000			
We	-0.0162	-0.0460	-0.0461	-0.0301	-0.0447	-0.0408	-0.0515	-0.5804	-0.0427	0.0000		
Wr	-0.0243	-0.0420	-0.0501	-0.0362	-0.0483	-0.0454	-0.0507	-0.5559	-0.0451	-0.0395	0.0000	
Y	-0.0139	-0.0273	-0.0462	-0.0449	-0.0515	-0.0405	-0.0444	-0.5112	-0.0254	-0.0406	-0.0406	0.0000

Figure 1. Structure analysis based on 14520 SNPs across 10 Chinese populations, with *K* = 6. Each bar represents one individual and the colours of the bars the posterior probability that each belongs to one of the six genetic clusters.

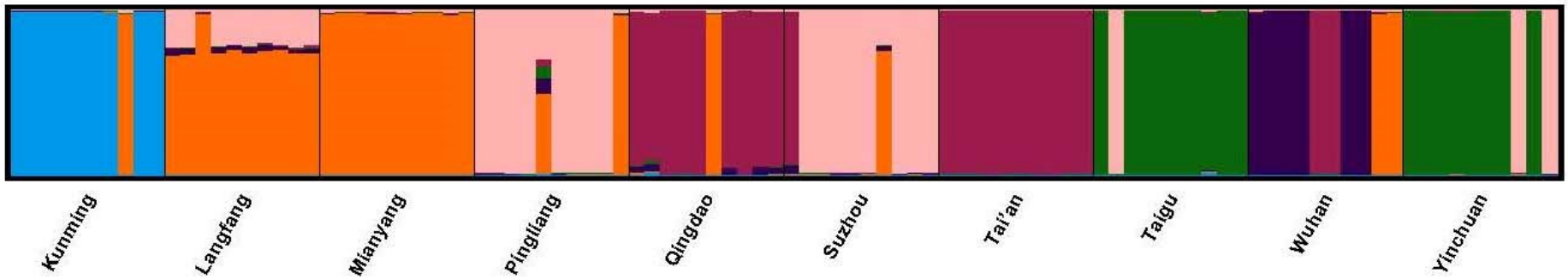
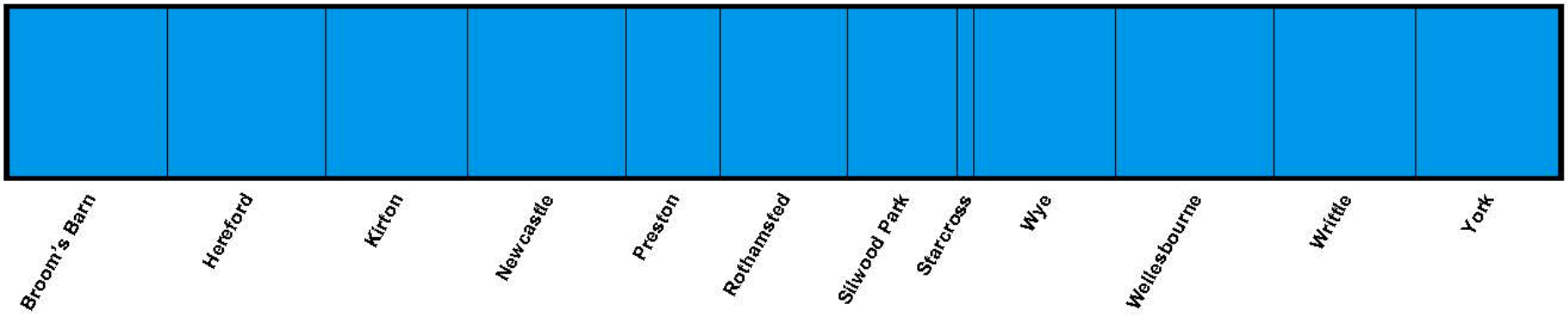


Figure 2. Structure analysis based on 846 SNPs across 12 English populations of *S. avenae*, with K = 1 (a) and K = 2 (b). Each bar represents one individual and the colours of the bars the posterior probability that each belongs to each of the genetic clusters.

a)



b)

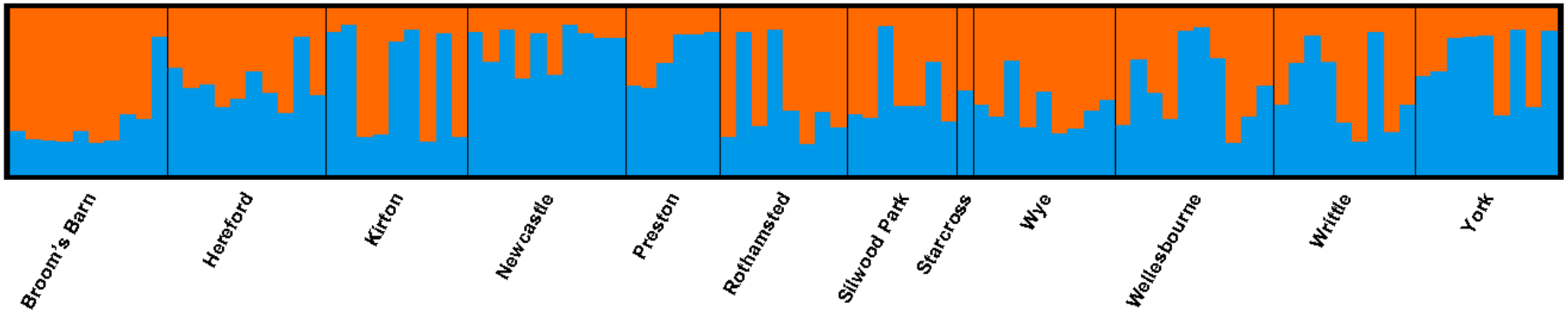


Figure 3. Midpoint rooted phylogenetic tree estimated with RAxML for the *S. avenae* samples from England using a dataset of 846 SNPs. The two multi-marker haplotypes from every individual are coloured in red and green, and the clades have been collapsed except for the earliest-branching haplotype of each clade. Labels on branches are bootstrap values > 90%.