# GENETIC DIFFERENTIATION IN POPULATIONS OF THE CORN LEAF APHID *RHOPALOSIPHUM MAIDIS* (FITCH) HEMIPTERA: APHIDIDAE

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## ABSTRACT

**The corn leaf aphid is a major pest causes damage to a range of cultivated crops. Little is known about the population genetics of the species. In this experiment, we examined the population genetic structure of the *Rhopalosiphum maidis* based on nucleotide sequencing of the cytochrome oxidase I (COI) gene and microsatellite analysis. Thirteen microsatellite markers developed for related aphid species were tested for use with *R. maidis*. Nine loci were found to amplify in *R. maidis*. Of these, seven were polymorphic. Nucleotide sequencing showed low variation, with one haplotype dominating in the regions sampled and four other haplotypes, differing by only a single base, occurring at a low frequency. Microsatellites confirmed low levels of diversity, but also showed moderate levels of population structure over local geographic scales, although this structure was not a result of isolation by distance. Over broader geographic scales, cluster analysis showed that populations from all U.S. states and China were almost indistinguishable and population assignment revealed that only 30% of individuals sampled could be correctly assigned to the population from which they were collected. Populations collected from *Sorghum bicolor* and *Sorghum halepense* could not be discriminated, indicating that *S. halepense* may act as a reservoir for the aphid during times when sorghum is not cultivated. The close similarity between individuals from China and the U.S. suggests that the lack of variation in the species may be explained by the parthenogenetic mode of reproduction, where males are seldom found.**

**Keywords: Microsatellite DNA, cytochrome oxidase I, sorghum, Johnsongrass, parthenogenesis**



## التمايز الوراثي في مجتمع حشرة من أوراق الذرة *Rhopalosiphum maidis* (Fitch) Hemiptera: Aphididae

**اليشيا ايفا تيم لؤي قحطان خلف كارلوس مايكل سمث**
**باحث علمي أستاذ أستاذ متميز**

### المستخلص

**تُعد حشرة مَنْ أوراق الذرة من الآفات الرئيسية التي تسبب أضرارًا لمجموعات كثيرة من المحاصيل. المعلومات المتوفرة عن الوراثة السكانية لهذه الحشرة قليلة. قمنا بفحص التركيب الوراثي السكاني لـ *Rhopalosiphum maidis* بناءً على تسلسل النيوكليوتيدات cytochrome oxidase I (COI) و تحليل microsatellite. تم اختبار ثلاثة عشر microsatellite markers التي تم تطويرها لأنواع المَنْ. اذ تم اكتشاف تسعة مواقع قابلة للتضخيم في حشرة *R. Maidis*. سبعة من هذه المواقع كانت متعددة الأشكال. أظهر تسلسل النيوكليوتيدات تبايناً منخفضاً، اذ وجد نمط حيوي واحد فقط في المناطق التي تم أخذ العينات منها، كما تم ملاحظة أربعة أنماط حيوية أخرى تختلف بقاعدة نايتروجينية واحدة فقط. أكد تحليل microsatellite بان هناك مستويات منخفضة من التنوع، كما وأظهرت مستويات معتدلة من التوزيع السكاني في مستوى النطاق الجغرافي الصغير. أما على مستويات النطاقات الجغرافية الأوسع، فقد أظهر النتائج أنه لا يمكن التمييز بين العينات التي جمعت من الولايات الأمريكية والصين، كما وكشفت النتائج أن 30٪ من العينات يمكن ان تدل على مجتمع الحشرات الاصلي الذي تم الجمع منه. لا يمكن التمييز بين المجموعات التي تم جمعها من الذرة البيضاء و عشبة جونسون، مما يشير إلى أن هذه ال عشبة بمثابة مستودع لحشرة المَنْ خلال الأوقات التي لا تتم فيها زراعة الذرة. و يشير التشابه الوثيق بين الأفراد التي تم جمعها من الولايات المتحدة و الصين إلى أن عدم وجود تنوع في الأنواع يمكن تفسيره من خلال نمط التكاثر العذري، حيث نادرًا ما توجد الذكور.**

**الكلمات المفتاحية: Microsatellite DNA، COI ، الذرة البيضاء، عشبة جونسون، التكاثر العذري**

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## INTRODUCTION

The corn leaf aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) is present on all continents where agricultural production is widespread. The aphid originated in Asia, and is now widely distributed throughout the U.S., where it was first recorded in 1856 (4). Although the primary host of this species is corn *Zea mays*, alternative hosts include other Poaceae such as sorghum *Sorghum bicolor* and barley *Hordeum vulgare*, other widely cultivated crops in the Solanaceae and Caricaceae, and many weed species. Karyotype analysis has shown that populations collected from corn, sorghum and Johnsongrass *S. halepense* have a 2n = 8 karyotype whereas those from barley and barnyard grass *Echinochloa crus-galli* have a 2n = 10 karyotype (5). Other than these results, no genetic evidence of host races have been confirmed although it has long been suggested that biotypes of *R. maidis* associated with different hosts exist, including on different sorghum varieties (27). The question of whether *R. maidis* has formed biotypes on different sorghum varieties is interesting given the widespread cultivation of *S. bicolor* and the cosmopolitan distribution of other sorghum species that are largely regarded as weeds. One of these weed species, *S. halepense*, is known to display greater resistance to insect feeding. Thus, one of the objectives of this study was thus to determine whether *R. maidis* has formed host races on cultivated *S. bicolor* and uncultivated *S. halepense*, based on an analysis of the population genetic structure of the species (43). Using molecular biology nowadays is one of the keystones (18, 29) to save time (35) in understanding the evolution and biodiversity (1, 2). Several genetic markers have been used to analyze the population genetic structure of *R. maidis* (23, 31) used restriction enzyme endonucleases and a ribosomal DNA probe and found that these markers were sufficient to distinguish among five clones collected from Johnsongrass across the southern U.S., although a low sample number was used. (37) examined karyotypic, allozyme and restriction enzyme analysis of the mitochondrial ND1, and portions of the 16S and CytB genes of populations collected from Spain, France, Morocco, two Canadian provinces (Ontario and Quebec), and four states in the U.S. (Illinois, Idaho, Washington and Nebraska). Although chromosome number varied according to host use, neither allozyme nor mtDNA restriction enzyme analysis allowed clonal discrimination. Since these studies were published, molecular markers that are capable of detecting higher levels of genetic variation that are more suited for population genetic analysis are commonly applied. Nucleotide sequencing of the mitochondrial cytochrome oxidase I (COI) gene is most commonly used for identification of arthropods based on the DNA barcoding procedure (13, 16, 17). This gene also has some use in population genetic studies, and the ready availability of aphid nucleotide sequences available in public databases makes this marker attractive for such studies (25, 30). In addition, since the COI gene may be used to identify aphids (12), its use prevents the unintentional inclusion of related aphid species in analyses. One other marker that has found the most application in studies of aphid population genetics in aphids (19, 22, 24), is microsatellite DNA. Microsatellite loci are highly polymorphic and noncoding and thus suitable for determining the genetic structure of populations and their demographic history as variations are independent of natural selection (41). One of the limitations of using this marker is that the identification of these regions is often labor intensive, costly, and species-specific. However, microsatellite markers developed for aphids may sometimes cross-amplify in related species (29, 30). Thus, another objective of this study was to determine whether microsatellite markers developed for related species could be used for *R. maidis* populations. Estimates of population differentiation and gene flow can provide insight into the factors such as historical processes and dispersal that shape population structure. Thus, analyzing the population genetics of a species may assist with managing its population levels by determining appropriate strategies for pest control. In addition to analyzing the genetic structure of *R. maidis* to determine whether host races have developed on *S. bicolor* and *S. halepense*, we also investigated the influence of geography on structuring populations. We concentrated

on Kansas, USA specimens to examine variation over local scales. We also included samples from Missouri, Oklahoma, Arizona and New Mexico (USA) to determine variation over larger geographic scales and determine whether populations in the Midwest U.S. were structured. In addition, populations from four Chinese populations were included to determine how levels of variation in populations from the native range compared to those in its introduced range.

## MATERIALS AND METHODS

### Sample collection, Insect material

Aphids were collected from Kansas, Missouri, Oklahoma, Arizona and New Mexico (Fig. 1, Table 1). The majority of specimens were collected from Kansas to examine variation over local scales. Populations were sampled from 14 counties, with three or more populations sampled from six of these. Specimens were collected from sorghum and Johnsongrass in Kansas and from only Johnsongrass in Missouri, Oklahoma, New Mexico and Arizona. Chinese *R. maidis* specimens were included as a means of comparing U.S. populations to those in its native range. These were collected from corn in Shaanxi, Heilongjiang, Liaoning and Fujian provinces. Specimens were collected during three years in USA and China. Specimens were collected in ethanol and stored at -80$^{o}$C until DNA extraction. A subsample of U.S. populations was included in nucleotide sequence analysis since low variation was expected for this marker, which was primarily included for aphid identification purposes as several related aphid species feeding on sorghum closely resemble *R. maidis*. Sequences were mined from the Barcode of Life Database (BOLD) as well as from GenBank to determine relationships from a greater geographic area than the U.S. Midwest and China. These sequences were obtained from individuals collected from Washington (HQ971338), Canada (EU701890, HQ970695, JF883915), India (HQ112195, JX051414, JX051396, JX051397, KP052755), Australia (DQ499048, DQ499049), the Philippines (KF022221), and Italy (KF639624). Larger sample numbers were analyzed from every population sampled using microsatellite markers (Table 1).

**DNA extraction:** Genomic DNA was extracted from whole aphids using the MyTaq™ Extract-PCR Kit (Bioline US) following (16). Aphids were lightly crushed with a sterile pipet tip before extraction to facilitate contact between the tissue and lysis buffer. Extracted DNA was stored at -80$^{o}$C until PCR.

**Nucleotide sequencing:** PCR was performed using standard DNA barcode procedures, with the primers LCO-1490/HCO-2198 (11). PCR amplification was carried out in a volume of 40µl, and included 0.5µl DNA, 2µl of each primer, 20µl MyTaq DNA Polymerase kit (Bioline) and 15.5µl sterile $H_2O$. All reactions were run under the following PCR program: 3 min at 95$^{o}$C, followed by 40 cycles of 95$^{o}$C for 20 s, 45$^{o}$C for 15 s and 72$^{o}$C for 20 s. A final hold step of 72$^{o}$C for 15 min completed the reaction. PCR products were visualized on agarose gels. Successful amplicons were bidirectionally sequenced by GeneWiz, Inc. using the amplification primers. Contigs were assembled from the forward and reverse read of each sample using Geneious version 9.1.7 (https://www.geneious.com). Using this software, sequences were aligned using MUSCLE (MUltiple Sequence Comparison by Log-Expectation; (9). Aligned sequences were used to confirm species identities with the identification engine on the Barcode of Life Data System (BOLD; boldsystems.org (31). All DNA sequences used in this study were uploaded to GenBank under accession numbers [PQ554715 - PQ554779]. Haplotype numbers were calculated using Arlequin v 3.5 (10) whereas haplotype networks were calculated with TCS (7) implemented in PopART (21).

**Microsatellite analysis:** Eight markers developed for *R. padi* [R1.35, R5.10, R2.73, R5.29b, R3.171, R5.138, R5.50, R6.3; (30)], two for *Acyrthosiphon pisum* [Apg20, ApEST41; 41)] and three for *Sitobion miscanthi* [SmS16b, S16b, S17b; (30)], were tested on a subset of *R. maidis* populations to determine whether they were able to amplify in this species.

**Table 1. Collecting details for specimens of *R. maidis* analyzed using COI sequencing and microsatellite analysis.**

| ID | Country | State | County/Region | *n*(Cs) | *n*(M a) | Town/City | GPS | Host |
|---|---|---|---|---|---|---|---|---|
| Rm4 | U.S.A | Kansas | Brown | - | 5 | Fairview | 39°50'26.9", -095°46'14.5" | Johnsongrass |
| Rm9 | | | Cherokee | - | 5 | Columbus | 37°15'18.4", -094°52'06.2" | Johnsongrass |
| Rm10 | | | | 2 | 5 | Columbus | 37°12'39.2", -094°52'13.8" | Sorghum |
| Rm12 | | | | - | 5 | Columbus | 37°12'45.7", -094°52'16.3" | Sorghum |
| Rm13 | | | | 2 | 5 | Columbus | 37°14'14. ", -094°51'13.9" | Johnsongrass |
| Rm44 | | | Ellis | 2 | 5 | Hays | 38°51'26.7", -099°20'09.0" | Sorghum |
| Rm61 | | | Finney | 2 | 5 | Garden City | 38°01'08.3", -100°46'26.9" | Sorghum |
| Rm63 | | | | 2 | 5 | Garden City | 37°59'57.1", -100°49'06.0" | Johnsongrass |
| Rm49 | | | Ford | - | 5 | Wright | 37°46'38.9", -099°54'22.4" | Sorghum |
| Rm58 | | | | - | 5 | Dodge City | 37°45'54.2", -099°59'10.8" | Sorghum |
| Rm3 | | | Labette | - | 5 | Parsons | 37°21' 12.4'', -095°17'24.1" | Johnsongrass |
| Rm8 | | | | 2 | 5 | Parsons | 37°21' 03.4", -095°19'20.8" | Johnsongrass |
| Rm14 | | | | 2 | 5 | Parsons | 37°21' 40.3", -095°17'37.7" | Sorghum |
| Rm73 | | | | 5 | 5 | Parsons | 37°21'39.0", -095°17'24.4" | Johnsongrass |
| Rm42 | | | Lincoln | - | 5 | Lincoln | 39°02’40.64", -98°100178" | Sorghum |
| Rm5 | | | Osage | 2 | 5 | Burlingame | 38°44'47.2", -095°49'42.3" | Johnsongrass |
| Rm16 | | | Riley | 2 | 5 | Manhattan | 39°13'32.1", -096°35'36.7" | Johnsongrass |
| Rm36 | | | | 2 | 5 | Manhattan | 39°12'45.7", -096°35'50.7" | Sorghum |
| Rm20 | | | Saline | - | 5 | Salina | 38°49'06.0", -097°42'53.0" | Sorghum |
| Rm28 | | | | - | 5 | Dayton | 38°54'34.0", -097°24'34.0" | Sorghum |
| Rm18 | | | Sedgewick | 2 | 5 | Garden Plain | 37°42'27.1", -097°41'33.5" | Sorghum |
| Rm30 | | | | 2 | 5 | Garden Plain | 37°42'21.9", -097°42'33.6" | Johnsongrass |
| Rm32 | | | | - | 5 | Garden Plain | 37°39'59.7", -097°41'52.7" | Johnsongrass |
| Rm66 | | | Shawnee | 2 | 5 | Topeka | 39°01'33.6", -095°56'48.3" | Sorghum |
| Rm48 | | | Smith | - | 5 | Smith Center | 39°45'22.0", -098°47'07.3" | Sorghum |
| Rm2 | | | Wabaunsee | 2 | 5 | Alma | 38°57'24.1", -096°16'21.7" | Johnsongrass |
| Rm72 | | Missouri | Cape Girardeau | 2 | 5 | Cape Girardeau | 37°32'33.6", -089°39'18.1" | Johnsongrass |
| Rm74 | | Oklahoma | Ottawa | - | 5 | Afton | 36°41'13.5", -094°58'41.4" | Johnsongrass |
| Rm75 | | | Washington | 2 | 5 | Bartlesville | 36°44'35.1", -095°56'47.7" | Johnsongrass |
| Rm83 | | Arizona | Cochise | 2 | 5 | Portal | 31°53'02.6", -109°12'22.8" | Johnsongrass |
| Rm84 | | New Mexico | Hidalgo | 2 | 5 | Animas | 31°59'57.6", -109°02'09.1" | Johnsongrass |
| Rm77 | China | Shaanxi Province | Yangling region | 6 | 6 | | 34°16'09.5", -108°04'25.9" | Corn |
| Rm78 | | Heilongjiang Province | Haerbin region | 6 | 6 | | 45°42'42.0", -126°54'24.1" | Corn |
| Rm79 | | Liaoning province | Shenyang region | 5 | 5 | | 41°49'34.1", -123°34'03.5" | Corn |
| Rm80 | | Fujian province | Ningde region | 5 | 5 | | 26°59'49.4", -119°25'11.5" | Corn |

***n*(Cs) = *n* (COI sequencing); *n*(M a) = *n* (Microsatellite analysis)**

**Fig. 1. Maps showing collecting locations of *R. maidis* for specimens analyzed in this study. Insets are shown of the U.S. Midwest, Kansas, and China to show collecting detail. Maps were created using SimpleMappr (34).**

Nine loci of these 13 loci amplified in *R. maidis*, and were used togenotype all populations sampled. Forward primers contained M13 tails to facilitate labelling with fluorescent dyes PET, 6-FAM, HEX and NED. PCR amplification for microsatellite reactions included 0.13µl DNA, 1µl of each primer (10 µM), 0.2ul of 10µM dye labelled M13 primer, and 5µl MyTaq DNA Polymerase kit (Bioline). All reactions were run under the following PCR program: 1 min at 95$^{o}$C, followed by 35 cycles of 95$^{o}$C for 15 s, 45$^{o}$C for 15 s and 72$^{o}$C for 10s. Analysis of fragment length was carried out by Genewiz, Inc (NJ, US) using a 3730xl genetic analyzer (Applied Biosystems, US). Genotypes were assigned using PeakScanner 2.0 (Applied Biosystems). Microchecker 2.2.3 (39) was used to check for scoring errors as result of stuttering, as well as null alleles and large allele dropouts. Linkage disequilibrium between loci was determined using Genepop 4.2 (27, 33). Multilocus FST and FIS values were also calculated using this software, as well as the number of migrants per generation (*N*m). In addition, this software was also used to test for departure from Hardy-Weinberg equilibrium (HWE) based on the hypothesis of heterogeneity deficit and excess. Observed (HO) and expected (HE) heterozygosities were also calculated using GenAlEx 6.5 (28). This software was also used to assess the correlation between genetic and geographic distance, to check for isolation by distance. The component variance of genetic differentiation among populations was determined based on analysis of molecular variance (AMOVA) and the fixation index PhiPT in GenAlex. Two different clustering methods were used to identify population structure. A neighbor-joining tree based on corrected FST distances and 10,000 bootstrap replications was constructed using PoptreeW (32, 38). Principal component analysis (PCoA) was performed to determine major patterns within the data set using GenAlEx. An assignment test was run to calculate the probability of each individual in each population belonging to a different population or to the population from which it was sampled. Population assignment was determined using GeneClass2 based on the frequencies-based method (29).

## RESULTS AND DISCUSSION

**DNA sequencing:** All species identities were confirmed using the Public Record Barcode Database identification engine on the BOLD System and found to match *R. maidis* specimens with at least 99% similarity based on sequence lengths of 621bp. A single haplotype was found for all specimens from the U.S. that were sequenced for this study, which included individuals from Kansas, Missouri, Oklahoma, Arizona and New Mexico. One individual from each Shenyang and Ningde regions in China showed a single base difference (in both cases an A-G substitution) to all remaining specimens, resulting in a total of three haplotypes for all sequences from four U.S. states and four

populations from China. The dominant haplotype was found at a frequency of 0.969. Sequences from GenBank were included in analyses to increase the data set, representing individuals from Washington, Canada, Italy, the Philippines, India, China and Australia. Specimens from Canada, Italy, China, and one sequence from India were the same dominant haplotype as that found in Kansas and China (Fig. 2). One individual each from the Philippines and Australia showed 1 bp difference to this dominant haplotype, although they were still part of the same haplogroup. The individual from Washington, one of the individuals from Australia, and three individuals from India showed differences of at least 6 bp from the dominant haplotype, and were clearly distinguishable from all remaining samples, forming a second haplogroup. The addition of GenBank data thus resulted in five additional haplotypes.

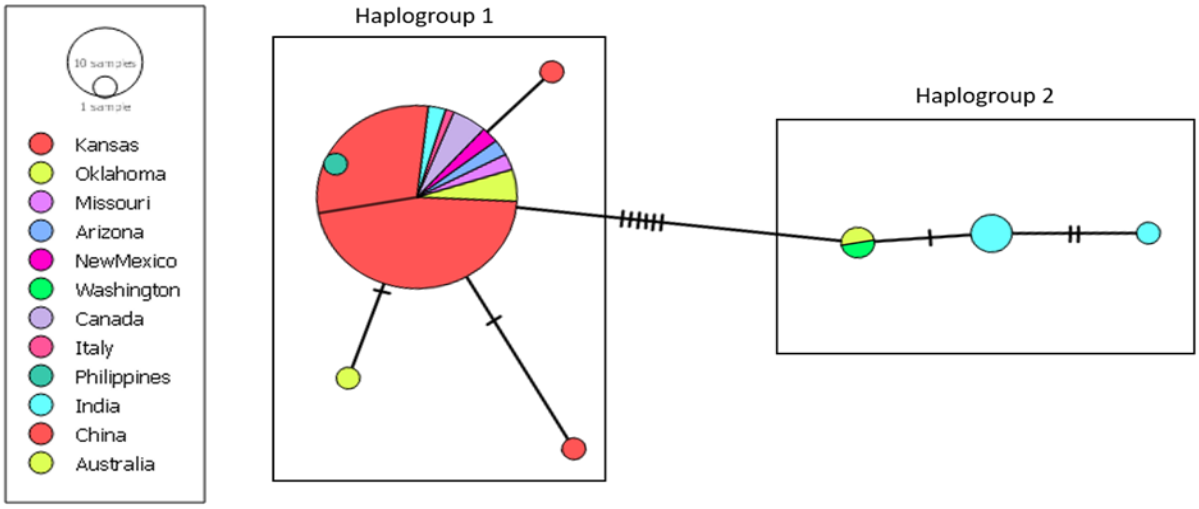


**Fig. 2. Median-joining network showing the relationships among mitochondrial haplotypes in *R. maidis* collected from the U.S., Canada, Italy, the Philippines, India, China and Australia. Circles represent different haplotypes and are colored to correspond with the sample population and scaled according to the number of supporting sequences. Hashed lines indicate the number of mutational steps between haplotypes**

**Microsatellite analysis**

**Marker testing:** Of the 13 microsatellite markers that were tested, four failed to amplify consistently, or to amplify at all, even at different annealing temperatures. The remaining nine loci were used for amplification of all populations. Of these nine loci, two (Apg20 and R5.10) were monomorphic and showed no variation for all specimens analyzed. Table 2 shows the nine loci that amplified in *R. maidis*. There was no evidence of large allele dropout or null alleles in any of the loci that were amplified and linkage disequilibrium between pairs of loci was not significant.

**Table 2. Microsatellite loci that amplified in *R. maidis*, out of a total of 13 loci that were evaluated**

| Locus | Alleles | Size Range | $H_O$ | $H_E$ | Species for which primers were designed | Reference |
|---|---|---|---|---|---|---|
| **Apg20** | **1** | **152** | **0** | **0** | ***Acyrthosiphum pisum*** | **(41)** |
| **R5.10** | **1** | **270** | **0** | **0** | ***R. padi*** | **(36)** |
| **R2.73** | **3** | **301-317** | **0.533** | **0.414** | ***R. padi*** | **(36)** |
| **R5.29b** | **3** | **191-201** | **0.967** | **0.505** | ***R. padi*** | **(36)** |
| **R5.138** | **5** | **145-157** | **0.006** | **0.060** | ***R. padi*** | **(36)** |
| **R3.171** | **4** | **269-297** | **0.985** | **0.569** | ***R. padi*** | **(36)** |
| **R5.50** | **3** | **155-159** | **0.067** | **0.087** | ***R. padi*** | **(36)** |
| **S16b** | **4** | **167-185** | **0.992** | **0.523** | ***S. miscanthi*** | **(42)** |
| **S17b** | **3** | **165-171** | **0.038** | **0.088** | ***S. miscanthi*** | **(42)** |

Polymorphism was low across all loci, and the seven polymorphic loci each had between three and five alleles. Locus R5.29b was only polymorphic when Chinese samples were included. Three loci were monomorphic in Kansas, five in Missouri and Oklahoma, three in New Mexico, two in Arizona and five in China. Three loci in Kansas (R5.29b, R3.171 and S16b) and one in China (S16b) deviated from Hardy-Weinberg equilibrium (P<0.001) because of heterozygote excess.

**Microsatellites**

**Variation over local scales (Kansas):** Fifteen localities in Kansas were sampled to obtain an estimate of population variation over local scales. AMOVA analysis showed that 32% of the variation was a result of variation among populations, with a PhiPT value of 0.317 (P<0.001). Pairwise population FST values ranged from 0, indicating that populations were indistinguishable, to 0.21, indicating moderate amounts of diversity (Table 3). After correcting for population size, the number of migrants between populations in Kansas was calculated as 1.45329. PCoA analysis showed that there was little pattern to population clustering (data not shown), and Mantel tests indicated a non-significant correlation between genetic and geographic distance ($R^2 = 0.0495$). Heterozygote excess was recorded in 8 out of the fifteen populations sampled in Kansas - Parsons, Burlingame, Columbus, Manhattan, Garden Plain, Salina, Garden City and Topeka. Correspondingly, low FIS values were recorded for populations (Table 4), with the total FIS value for Kansas calculated as -0.702. Variation among *R. maidis* populations within localities in Kansas was evaluated by sampling multiple populations from the same localities in Garden City, Manhattan, Parsons, Garden Plain and Columbus. Garden City and Manhattan populations were identical to each other (although only 2 populations were sampled for each). Of the three remaining localities, PhiPT values were significant only in Parsons (PhiPT = 0.792, P < 0.001).

**Variation among five U.S. states**

Cluster analysis showed little structure among populations from five U.S. states (Figs 3 and 4). AMOVA analysis indicated that 23.5% of the variation in U.S. states sampled was due to variation among states (PhiPT = 0.235, P < 0.001). FST values between populations collected from different states ranged from 0 (Kansas and Oklahoma, New Mexico and Arizona) to 0.0546 (Kansas and Arizona). The New Mexico population contained one private allele and the Arizona population contained three. Five loci were monomorphic in Missouri and Oklahoma, three in New Mexico and two in Arizona. FIS values ranged from 0 in Arizona to -1 in Missouri and Oklahoma (Table 4).

**Variation in Chinese populations**

Diversity in Chinese populations was low, and the four Chinese populations contained only four private alleles. These diversity measures were reflected in the mean number of alleles (2.556 in Kansas and 2.111 in China), as well as the proportion of heterozygotes (0.380 in Kansas and 0.357 in China). FIS values ranged from -0.27 in Ningde to -0.89 in Yangling, while the total FIS calculated for the four Chinese populations was -0.46. None of the four populations showed signs of heterozygote excess or deficiency. AMOVA analysis showed that 29% of the variation in Chinese populations was due to variation among populations (PhiPT = 28.7, P < 0.001). Principal co-ordinate analysis confirmed these results, and showed that Haerbin populations could be distinguished from other Chinese populations sampled (Fig. 5). This population was also more closely related to those from Kansas than from China (Fig. 4). Population assignment analysis, considering all U.S. and Chinese populations, showed that only 30.5% of the total number of individuals were correctly assigned to their own populations. The remainder could be assigned to other populations.

**Variation between hosts**

AMOVA analysis including individuals collected from sorghum and Johnsongrass indicated that only 1% of the variation could be ascribed to host use (PhiPT = 0.006, P = 0.206). Lack of population structure was confirmed by cluster analysis (Fig. 6). FST values between populations collected from Johnsongrass and sorghum was low (0.001). When corrected for population size the number of migrants between populations was 8.

**Table 3. Pairwise FST values for *R. maidis* populations sampled in Kansas.**

| | Alma | Parsons | Fairview | Burlingame | Columbus | Manhattan | Garden Plain | Saline | Lincoln | Hays | Smith Center | Wright | Dodge City | Garden City |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parsons** | **0.059** | | | | | | | | | | | | | |
| **Fairview** | **0.000** | **0.059** | | | | | | | | | | | | |
| **Burlingame** | **0.209** | **0.107** | **0.209** | | | | | | | | | | | |
| **Columbus** | **0.015** | **0.045** | **0.015** | **0.102** | | | | | | | | | | |
| **Manhattan** | **0.115** | **0.053** | **0.115** | **0.192** | **0.097** | | | | | | | | | |
| **Garden Plain** | **-0.003** | **0.062** | **-0.003** | **0.207** | **0.020** | **0.090** | | | | | | | | |
| **Saline** | **0.046** | **0.040** | **0.046** | **0.050** | **-0.002** | **0.010** | **0.050** | | | | | | | |
| **Lincoln** | **0.000** | **0.038** | **0.000** | **0.185** | **0.011** | **0.072** | **-0.006** | **0.036** | | | | | | |
| **Hays** | **-0.000** | **0.033** | **-0.000** | **0.130** | **-0.012** | **0.094** | **0.002** | **0.002** | **-0.006** | | | | | |
| **Smith Center** | **0.028** | **0.026** | **0.028** | **0.069** | **-0.015** | **0.094** | **0.029** | **-0.020** | **0.016** | **-0.016** | | | | |
| **Wright** | **0.007** | **0.016** | **0.007** | **0.112** | **-0.001** | **0.057** | **0.004** | **-0.002** | **-0.0096** | **-0.020** | **-0.019** | | | |
| **Dodge City** | **0.000** | **0.059** | **0.000** | **0.209** | **0.015** | **0.115** | **-0.003** | **0.046** | **0.000** | **0.000** | **0.028** | **0.007** | | |
| **Garden City** | **-0.001** | **0.072** | **-0.001** | **0.207** | **0.024** | **0.118** | **0.003** | **0.056** | **0.002** | **0.005** | **0.034** | **0.014** | **-0.001** | |
| **Topeka** | **0.000** | **0.059** | **0.000** | **0.209** | **0.015** | **0.115** | **-0.003** | **0.046** | **0.000** | **0.000** | **0.028** | **0.007** | **0.000** | **-0.001** |

**Table 4. FIS values for *R. maidis* populations collected from five U.S. states and four Chinese provinces.**

| State/Province | Location | FIS |
|---|---|---|
| Kansas | Alma | -1.0000 |
| | Parsons | -0.5699 |
| | Fairview | -1.0000 |
| | Burlingame | -0.8526 |
| | Columbus | -0.7355 |
| | Manhattan | -0.9524 |
| | Garden Plain | -0.8876 |
| | Saline | -0.6919 |
| | Lincoln | -0.8824 |
| | Hays | -0.7778 |
| | Smith Center | -0.7000 |
| | Wright | -0.7215 |
| | Dodge City | -1.0000 |
| | Garden City | -0.8885 |
| | Topeka | -1.0000 |
| Missouri | Cape Girardeau | -1.0000 |
| Oklahoma | Afton | -1.0000 |
| | Bartlesville | -0.7143 |
| New Mexico | Hidalgo | -0.6604 |
| Arizona | Portal | 0.0000 |
| Shaanxi Province | Yangling | -0.6807 |
| Heilongjiang Province | Haerbin | -0.8947 |
| Liaoning province | Shenyang | -0.4468 |
| Fujian province | Ningde | -0.2727 |

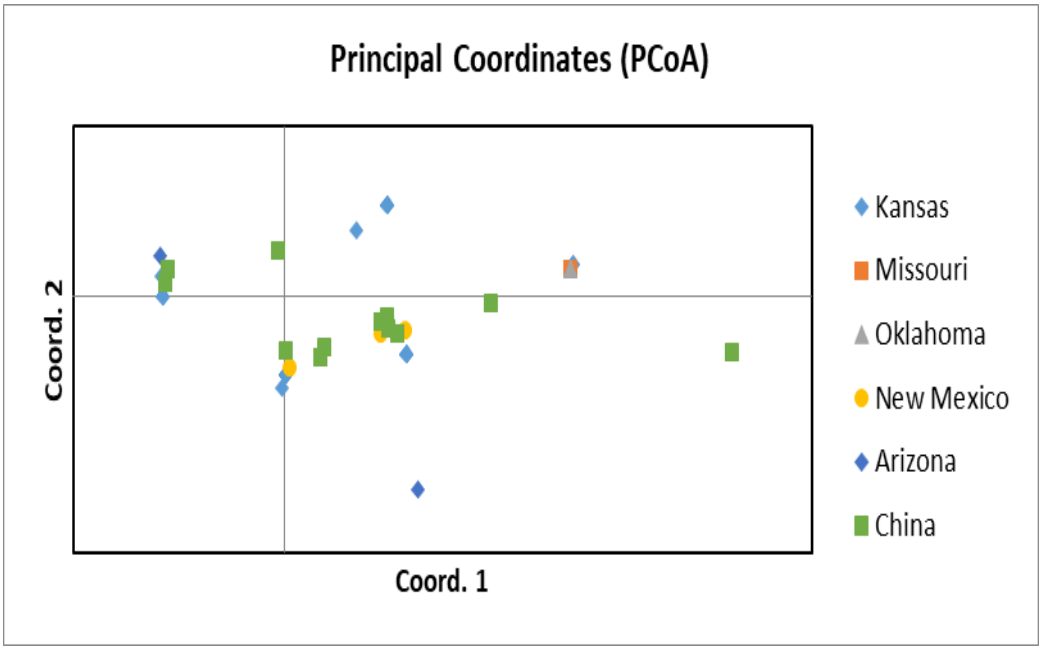


**Fig. 3. PCoA analysis showing little population differentiation among *R. maidis* populations collected form five U.S. states and four Chinese provinces**

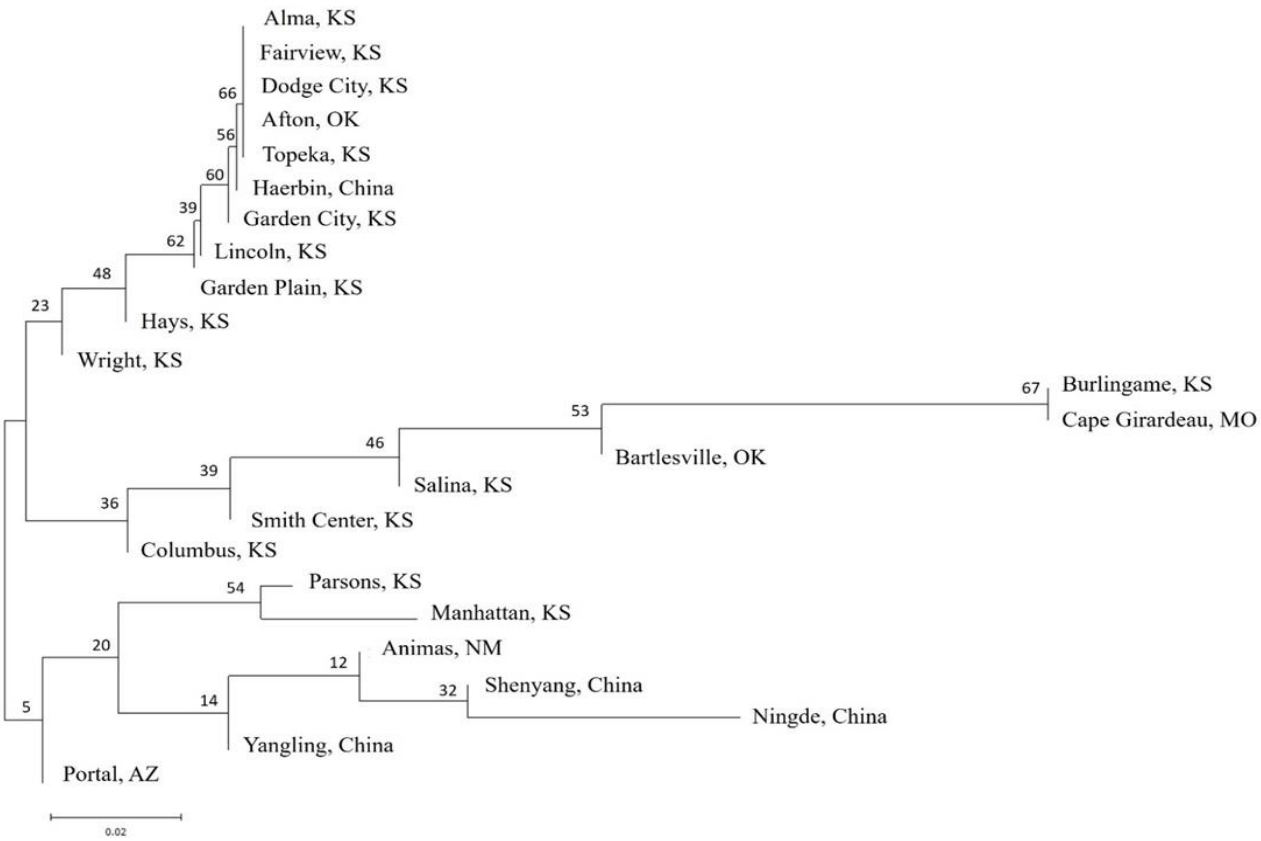


**Fig. 4. Neighbor-joining tree showing little differentiation among *R. maidis* populations collected from five U.S. states and four Chinese populations**

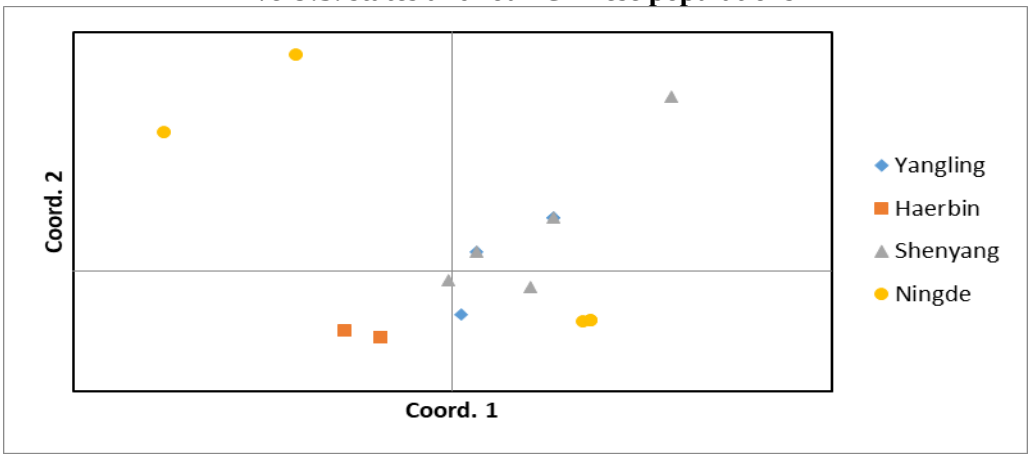


**Fig. 5. PCoA analysis showing variation in *R. maidis* populations collected from four regions in China**

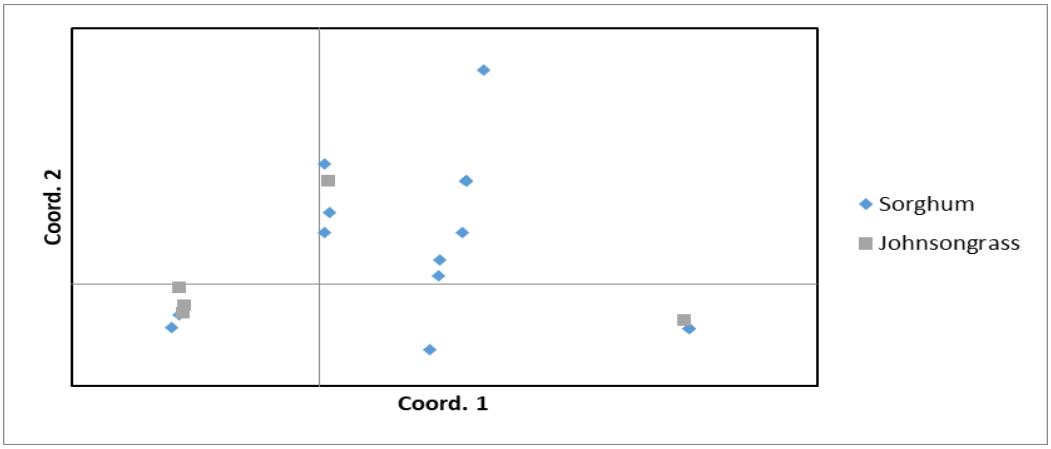


**Fig. 6. PCoA analysis showing little clustering in populations of *R. maidis* collected from sorghum and Johnsongrass**

**Sequence analysis:** Sequence analysis of the COI gene showed low variation within and among all *R. maidis* populations that were sequenced for this study. This includes individuals collected from five states from sorghum and Johnsongrass. When sequences from GenBank were included, individuals from Canada, Italy, the Philippines, and one specimen from Australia and India, were also shown to be almost indistinguishable from those sequenced for this study. However, one individual from Washington and Australia, and four from India clearly clustered separately to all other samples, forming a second haplogroup differing by at least 6 nucleotides from the primary haplogroup. Although host records were not available for all specimens from GenBank, the specimen from Australia was collected from barley, and the specimen from Washington from *Miscanthus giganteus*. It is known that *R. maidis* collected from barley and barnyard grass has a different karyotype (2n = 10) to those collected from wheat, corn, sorghum and Johnsongrass [2n = 8; (5)]. It is likely that the second haplogroup represents a different karyotype to those in the first haplogroup and that sequence analysis is sufficient to distinguish among karyotypes. However, sample sizes were small, and no known representative specimens of the 2n =10 karyotype were included in this analysis. Nevertheless, further investigation is justified since sequence analysis is more accessible for many molecular laboratories to diagnose *R. maidis* races than karyotype analysis.

**Microsatellite markers**

Microsatellite markers, because of their polymorphism, codominance and ease of use, are still one of the markers of choice for population genetics studies. However, their isolation remains time-intensive and expensive. Thus, microsatellite markers developed for related species are often tested for cross-amplification and have been found to be particularly applicable in aphids (30, 41). Our results show that microsatellite markers developed for related aphid species *R. padi*, *A. pisum* and *S. miscanthi*, in addition to microsatellite loci developed for this species.

***R. maidis* feeding on *S. bicolor* and *S. halepense:*** Populations of *R. maidis* collected from sorghum and Johnsongrass were not genetically distinct despite reports suggesting that different biotypes of the species had formed on these hosts in Kansas (27). Thus, it appears as if aphids may move freely between sorghum and Johnsongrass. In the U.S. Johnsongrass is regarded as one of the most noxious weeds since it is very invasive and easily outcompetes native grasses. It is present in most regions in the U.S. where sorghum is cultivated and is often found at the edges of cultivated fields and along roadsides (25). The weed flowers from April to November in the U.S., although dates vary by location. The relatively long Johnsongrass growing season means that it is present at times of the year when sorghum is not cultivated. Since it may act as an alternate host for *R. maidis*, Johnsongrass may act as a reservoir for populations of this pest, and allow them to increase before sorghum is available for feeding. Johnsongrass is known to harbor biotype diversity in populations of *S. graminum* (6). This study shows that it is also an important source of inoculum for *R. maidis*.

**Parthenogenesis and *R. maidis***

The question of *R. maidis* reproductive mode is interesting, as the species was long thought to be exclusively anholocyclic and to reproduce only by parthenogenesis. The discovery of a sexually reproducing population in Pakistan (32) and subsequently also in Korea (20) associated with *Prunus* as a primary host challenged thinking about the reproductive mode of this species. Males have been observed, although they did not seem to mate, these observations suggest that the loss of sexual reproduction in *R. maidis* is relatively recent (15). The reproductive mode of a species has a significant effect on population genetics parameters. Parthenogenetic species usually deviate from Hardy-Weinberg equilibrium, as mutations are the only source of variation. Heterozygote excess is often a sign of parthenogenetic populations, along with negative FIS indices. However, only eight out of 15 *R. maidis* populations from Kansas and none of the four populations from China displayed heterozygote excess, although all populations had negative FIS indices. The absence of heterozygote excess in almost half of Kansas populations and all Chinese populations,

despite strict parthenogenesis thought to occur in these populations, may be explained by population size rather than the presence of sexual forms. It has been suggested that asexual aphid species may be able to delay the accumulation of mutations associated with parthenogenesis through an increase in population size, which enhances natural selection (15), which may explain the pattern of heterozygote excess seen here. Relative to the related but holocyclic *R. padi*, *R. maidis* did not seem to accumulate non-synonymous mutations as expected. This anomaly is thought to be due to the larger number of generations per year, which results in a more rapid evolutionary rate, which could prevent the accumulation of heterozygotes.

***R. maidis* overwintering**

Another factor that may account for low diversity observed is that a small number of migrants may have founded spring colonies after overwintering in the south, which it is known to do. However, it is not known whether populations may overwinter in Kansas, especially in years without very low temperatures. Overwintering small populations might survive in years without very low temperatures (8). It may be that only one or a few clones that overwintered in the south founded Kansas populations; this would account for low diversity.

**Dispersal and *R. maidis***

The low levels of genetic variation among populations, the high percentage of individuals that can be assigned to populations other than the one in which they were collected (70%), and the high number of calculated migrants among populations from Kansas (1.45) point to rates of dispersal in *R. maidis* substantial enough to ensure that populations do not become differentiated. Both alate and apterous forms of *R. maidis* can occur and their proportion can vary among years (26). Despite their size, *R. maidis* individuals are capable of sustained flights lasting longer than 6 hours (40), and have been found to migrate long distances (3). These migratory flights are often overnight and at high altitude (7, 40). In addition to dispersal by flight, it is likely that both alates and apterae are carried on wind currents, further increasing their migratory ability (8). Out of 26 populations sampled in Kansas in 2015 for this study, only three were found to contain alates. Thus, it seems likely that dispersal on air currents contributes to dispersal of the species to enable migration from earlier to later hosts (14), and to additional geographic regions.

## CONCLUSION

*Rhopalosiphum maidis* may move freely between sorghum and Johnsongrass, despite reports that biotypes of this species have developed on these hosts. Thus, removal of Johnsongrass in areas where sorghum is planted is justified, as the weed may introduce and increase populations of *R. maidis* at times when sorghum is not available. The dispersal ability of the species is significant despite the small size of the insects and should be taken into account as it may affect pest control practices such as insecticide resistance management.

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