Genetic structure of populations of *Aphis gossypii* (Hemiptera: Aphididae) on citrus trees in Northern Iran

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**Key words.** Hemiptera, Aphididae, *Aphis gossypii*, microsatellite, genetic differentiation, aphid, genotypic diversity, obligate parthenogenesis, Iran

**Abstract.** The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a serious pest of citrus in northern Iran, both because of the damage caused by its feeding and as a vector of several viruses. The genetic structure of populations of *A. gossypii* on citrus trees at eight localities in Iran was surveyed using seven polymorphic microsatellite loci. Of 240 individuals tested, 142 multilocus genotypes (MLGs) were identified. The presence of multicity genotypes and negative $F_{IS}$ values revealed that the major mode of reproduction in northern Iran is obligate parthenogenesis. The genotypic diversity of populations ranged between 0.24 and 0.93. Considerable genotypic diversity and a high frequency of unique MLGs, confirmed there is some cyclical parthenogenesis in the region. The analysis of molecular variance revealed high intrapopulation and weak interpopulation genetic differentiation (overall $F_{ST} = 0.036$) among the different populations. The UPGMA dendrogram of eight populations based on Nei’s genetic distance indicated two clusters: genotypes from West of Mazandaran and Guilan provinces and those from East of Mazandaran. The same results were also obtained from the STRUCTURE analysis of these populations. This information on the genetic diversity of populations of *A. gossypii* in northern Iran could be useful for improving the Integrated Pest Management of this aphid.

**INTRODUCTION**

During the last four decades, the use of molecular markers has provided new information about the biology of aphids including their phylogeny, life-cycles, host-plants and geographical dispersal. The greatest contribution of these markers has been in the genetic differentiation among and between populations (Loxdale & Lushai, 2007; Loxdale et al., 2017). Generally, aphids have two modes of reproduction: obligate parthenogenesis and cyclical parthenogenesis with a single sexual generation that produces the over-wintering diapausing eggs (Dixon, 1998). However, some species of aphids have only one overwintering strategy and others overwinter both anholocyclically and holocyclically (Dedryver et al., 1998).

The cotton-melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a cosmopolitan species distributed worldwide (van Emden & Harrington, 2007). It is an important pest of various plant families, such as Rutaceae, Cucurbitaceae, Malvaceae, Solanaceae and Rosaceae (Ebert & Cartwright, 1997; Razmjou et al., 2006). It causes direct damage by feeding on sap and excreting honeydew and also indirect damage by transmitting several pathogen-ic plant viruses (Bertolini et al., 2000; Wang et al., 2016). There are two major citrus growing areas in Iran: (i) The northern part includes the Mazandaran and Guilan provinces in the Caspian Sea belt with a Mediterranean climate and periodically cold winters. These two provinces produce about 50% of the citrus fruit in Iran; (ii) The provinces in the southern region have tropical and subtropical climates (Bani Hashemian et al., 2013). *A. gossypii* is a serious pest of citrus orchards in northern Iran (Rassouliaian et al., 2001), with cotton, cucumber, pumpkin and hibiscus as the other hosts of this aphid (Razmjou et al., 2010; Kheyrollahi et al., 2013). Moreover, it is a vector of *Citrus tristeza virus*, the most important viral pathogen of citrus (Moreno et al., 2008), in the region (Alavi et al., 2000). Citrus yellow vein clearing virus and Citrus vein enation virus were also recently reported in this area (Nouri et al., 2016; Bani Hashemian & Aghajanzadeh, 2017). These pathogens can be transmitted by *A. gossypii* (Vives et al., 2013; Zhou et al., 2015). There are many diverse kinds of natural enemies in the citrus orchards of northern Iran, so little insecticide is used to control aphids in this region (Aghajanzadeh et al., 1997).
microsatellite markers is a useful tool for genomic charac-
terization of the soybean aphid, *Aphis glycines* Matsumura
and the cabbage aphid, *Brevicoryne brassicae* (L.) from
different geographic regions (Ruiz-Montoya et al., 2003;
Michel et al., 2009; Jun et al., 2011). In addition, the ge-
netic structure of populations of the Spirea aphid,
*Aphis spiraecola* Patch on pear trees in 13 provinces in China
has revealed high levels of genetic exchange facilitated by
geography and climate (Cao et al., 2012). In other similar
studies, molecular markers are used to demonstrate migra-
tion and dispersal in several other pest species of aphids
(Delmotte et al., 2002; Llewellyn et al., 2003; Lushai &
Loxdale, 2004).

In the present study, a suite of polymorphic microsatel-
lite markers was used to study the genetic diversity and ge-
netic structure within and among populations and thereby
deduce the life cycle of the *A. gossypii* that infest citrus
trees in the north of Iran.

**MATERIAL AND METHODS**

**Sample collection**

Aphid samples were collected from different commercial citrus
orchards at eight locations in the Guilan and Mazandaran prov-
inces in northern Iran (Fig. 1). Thirty individuals were collected
within an area of approximately 10 km² at each location in 2015
and 2016 (Table 1). Aphids from a single tree were considered as
one sample and only one aphid per sample was analyzed. Aphids
were taken from trees that were at least 50 m apart from each
other and the distance between any two populations was > 50 km.
Aphids were stored in micro tubes in 75% ethanol at 20°C prior
to DNA extraction.
DNA extraction and microsatellite analysis

DNA was extracted from one aphid using the cetyltrimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle, 1987). The DNA was diluted 10-fold and stored at –20°C. PCR was performed using 10 μL of the reaction mixture, containing 6 μL of 2× PCR Master Mix (Jena BioScience GmbH, Jena, Germany), 1 μL (10 pM) of each forward and reverse primer (Ago89, Ago66, Ago24, Ago59, Ago53, Ago84, Ago69, Ago126) (Vanlerberghe-Masutti et al., 1999) and 1.5 μL of aphid template DNA (approximately 10 ng), using the following cycling parameters: initial denaturation at 94°C for 1 min followed by 35 cycles of denaturation at 94°C for 5 min and extension at 74°C for 5 min.

Products were separated on 6% polyacrylamide urea gel at 75 W constant power (Bio-Rad Laboratories, Hercules, CA, USA). DNA fragments were visualized using ethidum bromide staining with the standard DNA Ladders SM1203 and SM1153 (FernberLoumat, France). Allele sizes were determined by comparison and documented using the UV DOC system (EBOX VX5/20, Vilentis, Waltham, MA, USA).

Data analysis

The GenClone v.2.0 program (Arnaud-Haond & Belkhir, 2007) was used to identify different multilocus genotypes (MLG), calculate genotypic diversity (R), defined as \((G−1)/(N−1)\), where G is the number of different MLGs and N is the number of individuals in a population and \(P_{loc}\), the probability of replicate MLGs being produced by independent sexual events. Calculation of the number of alleles (N), the effective number of alleles (\(N_e\)), F-statistics and Hardy-Weinberg Equilibria (HWE) was done using GENAIEX V 6.5 (Peakall & Smouse, 2012). To analyze deviations from HWE, only a single representative of each multilocus genotype (MLG) was considered. Also, analysis of Molecular Variance (AMOVA) was calculated using this program in order to determine differences within and among populations. A Mantel test of isolation by distance (IBD) with 1,000 permutations was run in GENAIEX. MICRO-CHECKER software (van Oosterhout et al., 2004) was used to test for the possible occurrence of null alleles at the microsatellite locus tested. Genetic distance was calculated and a UPGMA dendrogram depicted using POPGENE 32 (Yeh et al., 1999).

STRUCTURE program version 2.3.4 (Pritchard et al., 2000) was used to infer the structure of the population and estimate the most likely number of distinct population clusters. The number of clusters (K) considered was from 1 to 10, and 5 replications with a burn-in length of 500,000 iterations and a Markov chain of 500,000 steps were executed. The most likely number of genetic clusters based on the log probability of the data was calculated using the method of Evanno et al. (2005).

Table 1. Summary of the information and genetic variability revealed by using seven microsatellite loci and Aphis gossypii collected from eight populations infesting citrus trees in northern Iran.

<table>
<thead>
<tr>
<th>Sample collected from</th>
<th>Rezvanshahr - Guilan</th>
<th>Khoramabad - Mazandaran</th>
<th>Chaloss - Mazandaran</th>
<th>Rudsar - Guilan</th>
<th>Noor - Mazandaran</th>
<th>Sari - Mazandaran</th>
<th>Gluogah - Mazandaran</th>
<th>Astara - Guilan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population code</td>
<td>REZ</td>
<td>KHO</td>
<td>CHA</td>
<td>RUD</td>
<td>NOO</td>
<td>SAR</td>
<td>GLU</td>
<td>AST</td>
</tr>
<tr>
<td>Coordinates</td>
<td>37°31´N/49°08´E</td>
<td>36°45´N/0°49´E</td>
<td>36°40´N/51°21´E</td>
<td>37°04´N/50°18´E</td>
<td>36°29´N/52°07´E</td>
<td>36°36´N/53°03´E</td>
<td>36°45´N/53°47´E</td>
<td>38°23´N/48°50´E</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>G</td>
<td>13</td>
<td>8</td>
<td>15</td>
<td>16</td>
<td>21</td>
<td>28</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>R</td>
<td>0.41</td>
<td>0.24</td>
<td>0.48</td>
<td>0.52</td>
<td>0.69</td>
<td>0.93</td>
<td>0.55</td>
<td>0.79</td>
</tr>
<tr>
<td>Repeated genotype</td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Significant (P_{loc})</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(N_e)</td>
<td>3.86</td>
<td>2.86</td>
<td>4.00</td>
<td>4.14</td>
<td>4.29</td>
<td>4.71</td>
<td>3.90</td>
<td>4.86</td>
</tr>
<tr>
<td>(H_o)</td>
<td>3.17</td>
<td>2.31</td>
<td>2.61</td>
<td>3.01</td>
<td>3.16</td>
<td>3.18</td>
<td>2.82</td>
<td>3.14</td>
</tr>
<tr>
<td>(H_e)</td>
<td>0.91</td>
<td>0.82</td>
<td>0.93</td>
<td>0.88</td>
<td>0.88</td>
<td>0.85</td>
<td>0.87</td>
<td>0.83</td>
</tr>
<tr>
<td>(H_o)</td>
<td>0.66</td>
<td>0.57</td>
<td>0.63</td>
<td>0.65</td>
<td>0.66</td>
<td>0.67</td>
<td>0.66</td>
<td>0.66</td>
</tr>
<tr>
<td>(F_{is})</td>
<td>-0.39</td>
<td>-0.45</td>
<td>-0.50</td>
<td>-0.36</td>
<td>-0.36</td>
<td>-0.29</td>
<td>-0.34</td>
<td>-0.28</td>
</tr>
</tbody>
</table>

N – no. of individuals per pop; G – multilocus genotype (clone sensu lato); R – clonal diversity; Repeated genotype – number of repeated genotypes per sample; Significant \(P_{loc}\) – number of clonal genotypes; \(N_e\) – observed no. of alleles per locus; \(N_e\) – effective no. of alleles per locus; \(H_o\) – observed heterozygosity; \(H_e\) – expected heterozygosity; \(F_{is}\) – fixation index.
RESULTS

Genic and genotypic diversity

Samples were genotyped using seven of the eight microsatellite primer pairs designed earlier by Vanlerbergh-Masutti et al. (1999). Locus Ago24 produced the same band pattern in all individuals and was hence excluded from the calculations. A total of 142 multilocus genotypes (MLGs) were distinguished in the 240 individuals. We refer to these as clones sensu lato (Loxdale, 2008). The number of repeated genotypes were calculated. A total of 142 multilocus genotypes (MLGs) ranged from 8 to 28 within each population. The lowest and highest genotypic diversity was recorded for KHO and SAR, respectively (Table 1). In the populations KHO, CHA and REZ, the genotypic diversity was ≤0.5.

Highly significant P<sub>st</sub> values indicate that most of repeated genotypes in the samples were members of the same clone. However, the number of repeated genotypes were assembled independently by sexual reproduction (Table 1). The observed and effective number of alleles per locus for the 8 populations of A. gossypii were not significant based on the matrix of pairwise F<sub>st</sub> values (Table 3). F<sub>st</sub> values showed a weak genetic structuring, while only 3 of 28 pairwise comparisons exceeded 0.05 (Table 3).

According to the IBD analysis, there was no significant correlation between linearized F<sub>st</sub> and geographic distance (r = 0.072, P = 0.71; Fig. 5) for the 8 populations. The minimum and maximum genetic distances were between the NOO and RUD populations (0.0012), and KHO and SAR populations (0.2031), respectively (Table 2). The UPGMA dendrogram of the 8 populations based on Nei’s genetic distance is depicted in Fig. 2. The GLU and SAR populations from East of Mazandaran clustered together and populations from the rest of Mazandaran (NOO, CHA and KHO) and Gilan (RUD, REZ and AST) were in another cluster.

The STRUCTURE analysis of A. gossypii populations estimated the log-likelihoods and the number of genetic clusters (K) (Evanno et al., 2005) (Fig. 3). These results indicate that the 8 populations group in two clusters: 97.08% of individuals could be assigned to one of the clusters with more than 80% probability and 2.92% were considered to be admixed with K = 2 (Fig. 4). Most genotypes collected from SAR and GLU were placed in cluster 1, while all genotypes from KHO and CHA, and most of those from the other localities were assigned to cluster 2 (Fig. 4).

Table 2. Nei’s unbiased genetic distance measures among eight populations of Aphis gossypii collected from citrus trees in northern Iran.

<table>
<thead>
<tr>
<th>Pop</th>
<th>REZ</th>
<th>KHO</th>
<th>CHA</th>
<th>RUD</th>
<th>NOO</th>
<th>SAR</th>
<th>GLU</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>REZ</td>
<td>****</td>
<td>*****</td>
<td>*****</td>
<td>****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>****</td>
</tr>
<tr>
<td>KHO</td>
<td>0.0484</td>
<td>****</td>
<td>*****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>*****</td>
<td>****</td>
</tr>
<tr>
<td>CHA</td>
<td>0.0603</td>
<td>0.0264</td>
<td>****</td>
<td>0.0164</td>
<td>0.0220</td>
<td>0.0199</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>RUD</td>
<td>0.0302</td>
<td>0.0779</td>
<td>0.0152</td>
<td>****</td>
<td>0.0121</td>
<td>0.0197</td>
<td>0.0132</td>
<td>0.0176</td>
</tr>
<tr>
<td>NOO</td>
<td>0.0342</td>
<td>0.1324</td>
<td>0.0731</td>
<td>0.0012</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>SAR</td>
<td>0.0321</td>
<td>0.2031</td>
<td>0.0164</td>
<td>0.0668</td>
<td>0.0562</td>
<td>****</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>GLU</td>
<td>0.0398</td>
<td>0.1683</td>
<td>0.1243</td>
<td>0.0340</td>
<td>0.0220</td>
<td>0.0199</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>AST</td>
<td>0.0420</td>
<td>0.1046</td>
<td>0.0714</td>
<td>0.0541</td>
<td>0.0215</td>
<td>0.0490</td>
<td>0.0476</td>
<td>****</td>
</tr>
</tbody>
</table>

Table 3. Pairwise F<sub>st</sub> values among the eight populations of Aphis gossypii collected from citrus trees in northern Iran.

<table>
<thead>
<tr>
<th>Pop</th>
<th>REZ</th>
<th>KHO</th>
<th>CHA</th>
<th>RUD</th>
<th>NOO</th>
<th>SAR</th>
<th>GLU</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td>REZ</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
</tr>
<tr>
<td>KHO</td>
<td>0.018ns</td>
<td>****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
</tr>
<tr>
<td>CHA</td>
<td>0.028ns</td>
<td>0.013ns</td>
<td>****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
</tr>
<tr>
<td>RUD</td>
<td>0.007ns</td>
<td>0.030ns</td>
<td>0.004ns</td>
<td>****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
</tr>
<tr>
<td>NOO</td>
<td>0.007ns</td>
<td>0.046ns</td>
<td>0.027ns</td>
<td>0.000ns</td>
<td>****</td>
<td>*****</td>
<td>*****</td>
<td>*****</td>
</tr>
<tr>
<td>SAR</td>
<td>0.006ns</td>
<td>0.074**</td>
<td>0.066*</td>
<td>0.024*</td>
<td>0.008ns</td>
<td>****</td>
<td>*****</td>
<td>*****</td>
</tr>
<tr>
<td>GLU</td>
<td>0.004ns</td>
<td>0.053*</td>
<td>0.043*</td>
<td>0.005ns</td>
<td>0.008ns</td>
<td>0.003ns</td>
<td>****</td>
<td>*****</td>
</tr>
<tr>
<td>AST</td>
<td>0.009ns</td>
<td>0.031ns</td>
<td>0.024ns</td>
<td>0.019ns</td>
<td>0.009ns</td>
<td>0.021*</td>
<td>0.017ns</td>
<td>*****</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, ns – not significant.
Table 4. Analysis of molecular variance used to compare the genetic variation in populations of *Aphis gossypii*, collected from citrus trees in northern Iran.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Among geographic populations</td>
<td>7</td>
<td>50,258</td>
<td>0.098</td>
<td>3%</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Within individuals</td>
<td>240</td>
<td>765,000</td>
<td>3.188</td>
<td>97%</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(B) Among 2 clusters inferred by STRUCTURE</td>
<td>1</td>
<td>93,300</td>
<td>0.529</td>
<td>14%</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Within individuals</td>
<td>233</td>
<td>745,000</td>
<td>3.197</td>
<td>86%</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

(A) Populations collected from citrus trees; (B) Two clusters according to Bayesian analysis.

**DISCUSSION**

In the present study, the genetic structure of populations of the aphid, *A. gossypii*, collected from citrus orchards at different localities in the Caspian Sea region of Iran was analyzed. The presence of multicopy genotypes, highly significant *P* _ns_ values in repeated genotypes and negative *F* _st_ values indicate that this aphid reproduces mainly by obligate parthenogenesis in the northern part of Iran (Table 1). *A. gossypii* is an obligate parthenogen in most parts of the world (Blackman & Eastop, 2000), but in some regions like North America, East Asia and China there are populations that are cyclically parthenogenetic (Zhang & Zhong, 1982; Blackman & Eastop, 2007). Previous investigations on the genetic diversity of populations of *A. gossypii* using eight microsatellite loci also indicate asexual reproduction, based on the nonrecombinant genotypes present in the populations (Fuller et al., 1999; Brévault et al., 2008; Charaabi et al., 2008; Chen et al., 2013). The same results are also reported for other species of pest aphids (e.g. Sunnucks et al., 1996; Miller et al., 2003; Vorburger et al., 2003; Vorwerk & Forneck, 2006; Aradottir et al., 2012).

Although we detected a clear genetic signature of clonal reproduction, existence of 142 different multilocus genotypes among the individuals and the number of non-significant *P* _ns_ values in single population indicate that some of the clones of this aphid in this region reproduced sexually (Table 1). Cyclical parthenogenesis can result in more genotypic diversity in populations of this aphid (Simon et al., 1996; Delmott et al., 2002; Wilson et al., 2002; Cao et al., 2012). Razmjou et al. (2010) report that samples of *A. gossypii* from cotton in the Caspian Sea region of Iran have a high genotypic diversity and many lineages on this host are cyclically parthenogenetic. A recent study in northern China showed considerable genotypic diversity among *A. gossypii* populations on cotton (Luo et al., 2016). Such studies are very valuable because they can be used to indirectly infer the type of life-cycle of an aphid (Le Trionnaire et al., 2008).

Whether or not aphids reproduce sexually or asexually depends on the winter climate (Rispe & Pierre, 1998). Sandrock et al. (2011) show that life cycle variation in the black bean aphid, *A. fabae*, is related to climate and aphids from areas with mild winters overwinter anholocyclically more than those from areas with cold winters. A mild winter in the Caspian Sea belt enables *A. gossypii* to overwinter parthenogenetically. Nevertheless, the occurrence of periodical cold winters seemingly induces some cyclical parthenogenesis and production of overwintering eggs in this region. When winters are cold, overwintering as sexually produced eggs is a dependable strategy because eggs in diapause are very cold-resistant (James & Luff, 1982; Rispe & Pierre, 1998; Simon et al., 1999).

Application of broad-range insecticides to host plants to control a pest can decrease the number of genotypes. In West and Central Africa, the increased use of insecticides on both cotton and other host plants (okra and roselle) has led to the prevalence of one genotype of *A. gossypii* resistant to different classes of insecticides (Brévault et al., 2011). In the Caspian Sea region, insecticides are rarely used to control citrus aphids, so the presence of many aphid genotypes is expected.

Results of the IBD analysis revealed that geographic distance had no effect on *A. gossypii* population structure. The AMOVA analysis identified a low level of population differentiation (Global *F* _st_ = 0.036) among populations of *A. gossypii* from citrus trees in northern Iran. Low genetic differentiation of *A. gossypii* was also indicated by both the UPGMA dendrogram based on Nei’s genetic distance and the STRUCTURE analysis. These high intrapopulation and weak interpopulation genetic diversity patterns may reflect significant gene flow among populations. The amount of gene flow based on the *F* _ST_ values support such a scenario. However, *N* _m_ (number of migrants) values varied greatly, ranging from 3.11 to 89.03 (data not shown). Our results are in agreement with the findings that low genetic differentiation confirms high gene flow. Little genetic differentiation among bird cherry-oat aphid (*R. padi*) populations in France, indicate extensive gene flow, at least over short distances (Delmotte et al., 2002). In a study on the peach-potato aphid, *Myzus persicae* (Sulzer) in Victoria, Australia, the low overall *F* _ST_ value recorded and the widespread occurrence of the two most common genotypes provided evidence that individuals of this aphid move relatively freely in that region (Vorburger et al., 2003).

In northern Iran, citrus orchards are situated along the Caspian Sea belt where there are no geographical barriers so aphids can move easily across this region. As a consequence, the lack of complete genetic separation of populations in this area is a reasonable deduction from our data. Aphids can migrate over long distances (Simon et al., 1999; Llewellyn et al., 2003), although, short-distance movements are generally more important for the distribution of genotypes in aphid populations (Lloyd, 1993). The genetic structure of *A. gossypii* collected from six localities in Tunisia confirmed that genetic differentiation among localities was not statistically significant (Charaabi et al., 2008), whilst a study of the genetic structure of sexual French populations of *R. padi* showed little geographical
differentiation among them, confirming the high dispersal ability of this aphid (Delmotte et al., 2002).

Based on the results of the STRUCTURE analysis, the SAR and GLU populations were grouped in one cluster and the other populations in another. The AMOVA also detected significant differences between these two clusters (Table 4). The east of Mazandaran, which includes the SAR and GLU populations has colder winters, warmer summers and less rainfall compared to other parts of the Caspian Sea region (Pirnia et al., 2015). Also, there are alternate hosts for A. gossypii there. The aphids on these hosts can also colonize citrus trees. So, the genetic differences between the two clusters may due to the above reasons. Further studies are required to determine the importance of these various factors. According to Cao et al. (2012), differences in climate in different regions should be considered when attempting to understand the population structure of A. spiraeola.

In conclusion, there is considerable genotypic diversity and high intrapopulation and weak interpopulation genetic differentiation among geographic populations of A. gossypii in northern Iran. Furthermore, A. gossypii on citrus, reproduces predominantly by obligate parthenogenesis in northern Iran. As indicated by this study and other studies on different species of aphids, assessment of population genetic diversity can be used in Integrated Pest Management and the findings used to develop more rational and sustainable methods of control. Lastly, based on the information gained during this research, it appears likely that further projects can be developed to study the relationship between the aphid’s genotype and virus transmission, such as that done earlier for M. persicae by Terrodot et al. (1999).

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