

# Plasticity in host utilization by two host-associated populations of *Aphis gossypii* Glover

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## Abstract

Biological and morphological plasticity in polyphagous insect herbivores allow them to exploit diverse host plant species. Geographical differences in resource availability can lead to preferential host exploitation and result in inconsistent host specialization. Biological and molecular data provide insights into specialization and plasticity of such herbivore populations. In agricultural landscapes, *Aphis gossypii* encounters several crop and non-crop hosts, which exist in temporal and spatial proximity. We investigated the host-specialization of two *A. gossypii* host-associated populations (HAPs), which were field collected from cotton and squash (cotton-associated population and melon-associated population), and later maintained separately in the greenhouse. The two aphid populations were exposed to seven plant species (cotton, okra, watermelon, squash, cucumber, pigweed, and morning glory), and evaluated for their host utilization plasticity by estimating aphid's fitness parameters (nymphal period, adult period, fecundity, and intrinsic rate of increase). Four phenotypical characters (body length, head capsule width, hind tibia length and cornicle length) were also measured from the resulting 14 different HAP × host plant combinations. Phylogenetic analysis of mitochondrial COI sequences showed no genetic variation between the two HAPs. Fitness parameters indicated a significant variation between the two aphid populations, and the variation was influenced by host plants. The performance of melon-aphids was poor (up to 89% reduction in fecundity) on malvaceous hosts, cotton and okra. However, cotton-aphids performed better on cucurbitaceous hosts, squash and watermelon (up to 66% increased fecundity) compared with the natal host, cotton. Both HAPs were able to reproduce on two weed hosts. Cotton-aphids were smaller than melon-aphids irrespective of their host plants. Results from this study suggest that the two HAPs in the study area do not have strict host specialization; rather they exhibit plasticity in utilizing several hosts. In this scenario, it is unlikely that host-associated *A. gossypii* populations would evolve into host-specific biotypes.

**Keywords:** plasticity, fitness, host specialization, alternate hosts

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## Introduction

Phytophagous insects often exhibit host specialization, where host-specialized populations assume greater fitness benefits on their natal hosts than on non-natal or novel hosts (Jaenike, 1990). In fact, even a majority of generalists are known to exhibit host specialization to some degree

(Futuyma & Moreno, 1988; Schoonhoven *et al.*, 2005; Sword *et al.*, 2005). Factors which may influence the degree of host specialization could be the genetic background of insect populations, local adaptation and experience (conditioning), differences in host availability in the landscape: spatially and temporally, nutritional quality of available host plants, and other ecological factors (Futuyma, 1976; Bernays & Chapman, 1994; McBride, 2007). Host specialization, even in generalists, could lead to evolution of host biotypes (Carletto *et al.*, 2009).

*Aphis gossypii* Glover (Hemiptera: Aphididae) is a cosmopolitan and polyphagous pest. It utilizes plants belonging to Asteraceae, Cucurbitaceae, Malvaceae, and Solanaceae (Ebert & Cartwright, 1997; Blackman & Eastop, 2000). *A. gossypii* exhibits considerable plasticity in host utilization, which allows it to use a variety of hosts in a landscape (Ebert & Cartwright, 1997; Wool & Hales, 1997; Bancroft, 2006). There are also numerous examples of host-specialized populations in the case of *A. gossypii*. For example, in Europe, *A. gossypii* from *Chrysanthemum* did not colonize cucumber and *vice-versa* (Guldmond *et al.*, 1994). Similarly in China, *A. gossypii* from cucumber did not survive on cotton (Gao & Liu, 2008). In addition to biological characterization of *A. gossypii* populations for their host specialization, several molecular studies have also revealed the existence of specialized host-adapted *A. gossypii* populations. Using microsatellite markers, Carletto *et al.* (2009) identified as many as five host biotypes (cucurbits, cotton, eggplant, potato, and chili) in *A. gossypii* populations collected from five different countries. The presence of multiple host biotypes, besides the commonly recognized cotton and cucurbit biotypes, was also documented by Gao & Liu (2008). Existence of various host biotypes in different geographical regions suggests that their evolution via host specialization may be contingent upon ecological characteristics, such as availability and composition of host and non-host plant species, host plant's phenological differences, climatic conditions, soil fertility, and agricultural practices (Funk & Bernays, 2001; Barman *et al.*, 2012; Stotz *et al.*, 2013).

Therefore, if host specialization is drastically influenced by the above-mentioned characteristics, then insect populations may not always follow the same pattern of host specialization throughout their distribution range (Fox & Morrow, 1981; Thompson, 2005). Different populations of the same herbivore species might exhibit specialization in some geographic locations while in other populations might be a generalist (Funk & Bernays, 2001; Gotthard *et al.*, 2004; Singer & McBride, 2012). In several geographic regions around the world, *A. gossypii* populations were found to represent distinct host-specialized populations as revealed by several genetic and biological studies (Guldmond *et al.*, 1994; Najar-Rodriguez *et al.*, 2009; Agarwala & Choudhury, 2013; Satar *et al.*, 2013). In the agricultural landscape of the southeastern United States, especially in Georgia, both cotton and several cucurbitaceous vegetables are commonly grown in temporal and spatial proximities. While random migration of *A. gossypii* individuals between cotton and cucurbits is usually assumed, existence of host-associated biotypes in this species would undermine that assumption.

In this study, we assessed host specialization and host utilization plasticity of two *A. gossypii* populations. The two *A. gossypii* host-associated populations (HAPs) were field collected and later maintained on cotton and squash for several generations in the laboratory. First, we characterized the two HAPs, one on cotton and another on squash, using

mitochondrial COI sequences. Later, fitness parameters of these two *A. gossypii* populations were evaluated on seven host plants; three host plants from Cucurbitaceae, two from Malvaceae, and two annual weed species belonging to two distant plant families (Amaranthaceae and Convolvulaceae). These plant species are typical representations of South Georgia farmscapes, where *A. gossypii* is highly prevalent and bound to encounter these selected plant species. Our objective was to measure the potential of the two HAPs in utilizing plant species other than their natal hosts. If HAPs cannot utilize alternate hosts, then there is a greater chance for that population to evolve into a distinct biotype. In contrast, presence of plasticity in host utilization in aphids would allow the population to feed opportunistically on an array of available hosts, and evolution of specialization will be constrained. Additionally, we evaluated phenotypical variations in two populations on their natal and alternate host species.

## Materials and methods

### Aphid populations

Approximately 100 *A. gossypii* adult apterae were collected from squash (*Cucurbita moschata* Duchesne ex Poiret) and cotton (*Gossypium hirsutum* L.) fields located at least 15 miles apart in Tifton, Georgia. These aphids were maintained separately on the originally collected host for multiple generations in greenhouse conditions (25–30°C and at 80–90% RH with a 14 h photoperiod). These two HAPs will be referred as either cotton-aphids or CAP and melon-aphids or MAP in this paper. The taxonomic identity of aphids, as *A. gossypii*, was confirmed by a hemipteran taxonomist, Dr Susan E. Halbert, at the Florida Department of Agriculture & Consumer Services in Gainesville, FL (voucher specimens: E2012-1467 and E2012-1470).

### Host plants

A total of seven host species, two Malvaceaeous hosts (cotton, *Gossypium hirsutum* L. and okra, *Abelmoschus esculentus* L.), three Cucurbitaceaeous hosts (watermelon, *Citrullus lanatus* (Thunb.), squash, *Cucurbita moschata* Duchesne ex Poiret, and cucumber, *Cucumis sativus* L.), and two non-crop hosts (pigweed, *Amaranthus palmeri* S. Wats., Amaranthaceae; and morning glory, *Ipomea alba* L., Convolvulaceae) were used. All crop species (cotton, okra, watermelon, squash and cucumber) were grown from commercially available seeds, and experiments were conducted when plants were at 3–5 true leaf stage. Weed species (pigweed and morning glory) were grown from field collected seedlings devoid of any prior aphid infestations. These host plants were selected because they are prevalent in the farmscapes of southern Georgia.

### Genetic diversity in aphid populations

In order to evaluate the genetic diversity of two host-associated (cotton and melon) populations of *A. gossypii*, four individuals from each population were randomly selected. Total DNA from individual aphids was extracted using Qiagen DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. A universal primer pair C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTC-3') and 12-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') was used to partially amplify the mitochondrial cytochrome

oxidase subunit 1 (COI) gene following the polymerase chain reaction (PCR) methods and conditions specified in Simon *et al.* (1994). Electrophoresis was conducted on a 1% agarose gel stained with ethidium bromide ( $0.05 \mu\text{g ml}^{-1}$ ). The amplicons were purified using Qiaquick PCR purification kit (Qiagen, Valencia, CA). The purified PCR products were sequenced in both directions (Eurofins MWG Operon, Huntsville, AL). Sequences from a total of eight individual aphids (GenBank® accession number: KC610670- KC610677), four from each HAP, were selected for phylogenetic analysis. COI sequences of two voucher specimens of *A. gossypii* (GU591547 and JQ860257) were also included in the analysis. *A. glycines* partial COI gene sequence (EF467229) was included as the outgroup taxon. The sequences were aligned using Clustal-X and corrected by sight after setting the parameters for pairwise alignment (gap opening = 10; gap extension = 0.10), and multiple alignment (gap opening = 10; gap extension = 0.20; delayed divergence = 30%) (Hall, 2001). Bayesian analysis was conducted in Mr. Bayes version 3.2 using the Monte Carlo Markovian Chain (MCMC) method (Ronquist and Huelsenbeck, 2003). The analysis used a general time reversible nucleotide substitution model with a gamma-distributed rate variation across sites along with a proportion of invariable sites. The phylogenetic tree was constructed using FigTree V1.4 (<http://tree.bio.ed.ac.uk/software/figtree/>).

#### Fitness parameters of *A. gossypii*

A pair of randomly selected apterous *A. gossypii* was placed using a camelhair paintbrush on the underside of a leaf of the respective host plants and confined with a clip cage. For each population, ten cages were placed on each host plant species. A total of 140 cages (2 HAPs  $\times$  7 host plants  $\times$  10 cages each) were placed. After 24 h of confinement, the females were removed, and only one freshly laid nymph was retained in each cage. The life history of this single aphid was monitored throughout the entire life cycle. For each HAP  $\times$  host plant combination, only surviving nymphs from 140 cages were considered for statistical analysis (Table 1). To measure total fecundity, the nymphs laid by each subsequent generation adult were counted and removed daily. Fitness parameters such as nymphal period, adult period and total fecundity were measured and intrinsic rate of increase was estimated. Nymphal and adult periods in this context refer to the duration in days from the first day of larviposition to the first day of adulthood, and from the first day of adulthood to the completion of life cycle, respectively. Total fecundity represents the number of nymphs produced by an apterate adult throughout its adulthood. The intrinsic rate of increase ( $r_m$ ) for each aphid was calculated using the equation of Wyatt & White (1977):

$$r_m = 0.747 \frac{\log_e N_d}{d}$$

where  $N_d$  is the number of nymphs produced during reproductive period, and 'd' is the pre-reproductive period in days. This experiment was repeated once with 10 cages for each host and each aphid population, thereby 280 (140  $\times$  2) cages were used altogether.

#### Phenotypical variation in *A. gossypii*

Approximately ten adults were placed on the undersurface of a leaf from seven different selected plant species. After 24 h, the adults were removed, and freshly laid nymphs were retained. These nymphs were allowed to feed on the respective host plants until they turned into adults, thus allowing completing an entire generation on the respective host plant. From each HAP  $\times$  host combination, 30 *A. gossypii* adults were collected on the first day of their adulthood and preserved in 70% ethanol. A total of 420 *A. gossypii* adults (2 HAPs  $\times$  7 host plants  $\times$  30 adults each) were individually slide mounted after processing the samples following the methods described by Blackman & Eastop (2000). Each slide mount was photographed under the microscope (40  $\times$  magnification). Four different phenotypical variables: body length, head capsule width (measured distance between eyes), hind tibia length, and cornicle length were measured using Olympus DP2-BSW software (Olympus America, Inc., Center Valley, PA).

#### Statistical analyses

Data from two repeats of the fitness experiment were pooled for statistical analyses. Effects of aphid populations and host plants on *A. gossypii* nymphal and adult periods were analyzed using median one-way analysis using PROC NONPAR1WAY in SAS Enterprise version 6.1 (SAS Institute, Cary North Carolina). *A. gossypii* fitness parameters *viz.* fecundity and intrinsic rate of increase, and phenotypical parameters were evaluated using a generalized linear mixed model (PROC GLIMMIX) in SAS. Aphid populations and host plants were considered as fixed effects and replications as random effects. Treatment means were separated using Fisher's Least Squares Difference at a 95% confidence level.

## Results

#### Genetic diversity in aphid populations

All the melon-aphids and cotton-aphids COI sequences in this study (Squash: KC610670-73; Cotton: KC610674-77) fell into a single *A. gossypii* clade. Mitochondrial COI sequences for the two HAPs in this study had maximum similarity with previously published *A. gossypii* COI sequences in the Genbank (GU591547, JQ860257, HQ528254, and FJ965680). This grouping was supported by 100% posterior probability value (Fig. 1). The MCMC tree also showed that all the *A. gossypii* were separated into one major clade from the out-group taxon, *A. glycines* (EF467229).

#### Fitness parameters of *A. gossypii*

##### Nymphal period

The nymphal periods of both HAPs significantly varied with host plants (CAP:  $\chi^2_{(1, 121)} = 100.9, P < 0.0001$ ; MAP:  $\chi^2_{(1, 112)} = 90.6, P < 0.0001$ ; Table 1). Unlike other host plants, both populations did not have significantly different nymphal periods on cucumber and pigweed. Cotton-aphids reared on squash (7.5 days) and watermelon (7 days) took longer to become adults than when reared on cotton (5 days) and okra (4.5 days). A similar trend was observed with melon aphids. They took longer to become adults when reared on cotton (5 days) and okra (6 days) than on watermelon (4 days). Individuals from both populations developed rapidly into adults on cucumber (cotton

Table 1. Host plant effects on developmental time and adult longevity of two *A. gossypii* HAPs.

Host plant:	N	Median development time (days)	Sum of scores	Expected under H0	SD under H0	Mean score	Statistics
<b>Cotton</b>							
<b>Nymphal period</b>							
Cotton-aphid*	20	5 (4–5)	7.15	10	0.91	0.35	$\chi^2_{(1, 30)} = 9.7$ $P = 0.0018$
Melon-aphid*	12	5 (5–7)	8.85	6	0.91	0.73	
<b>Adult period</b>							
Cotton-aphid	20	21 (19–22)	16	10	1.31	0.8	$\chi^2_{(1, 30)} = 20.7$ $P < 0.0001$
Melon-aphid	12	9 (8–11)	0	6	1.31	0	
<b>Okra</b>							
<b>Nymphal period</b>							
Cotton-aphid	20	4.5 (4–6)	2	9.71	1.41	0.1	$\chi^2_{(1, 33)} = 30$ $P < 0.001$
Melon-aphid	15	6 (6–8)	15	7.28	1.41	1	
<b>Adult period</b>							
Cotton-aphid	20	15 (14–17)	17	9.71	1.41	0.85	$\chi^2_{(1, 33)} = 26.3$ $P < 0.001$
Melon-aphid	15	9 (8–11)	0	7.28	1.41	0	
<b>Watermelon</b>							
<b>Nymphal period</b>							
Cotton-aphid	20	7 (6–7)	20	10	1.6	1	$\chi^2_{(1, 38)} = 39$ $P < 0.0001$
Melon-aphid	20	4 (4–5)	0	10	1.6	0	
<b>Adult period</b>							
Cotton-aphid	20	18 (17–20)	10.76	10	1.31	0.53	$\chi^2_{(1, 38)} = 0.34$ $P = 0.55$
Melon-aphid	20	18 (15–20)	9.23	10	1.31	0.46	
<b>Squash</b>							
<b>Nymphal period</b>							
Cotton-aphid	20	7.5 (7–8)	20	10	1.6	1	$\chi^2_{(1, 38)} = 39$ $P < 0.0001$
Melon-aphid	20	5 (4–5)	0	10	1.6	0	
<b>Adult period</b>							
Cotton-aphid	20	15 (13–16)	0	10	1.6	0	$\chi^2_{(1, 38)} = 39$ $P < 0.0001$
Melon-aphid	20	19.5 (17–21)	20	10	1.6	1	
<b>Cucumber</b>							
<b>Nymphal period</b>							
Cotton-aphid	17	4 (4–6)	6.26	8.27	1.23	0.36	$\chi^2_{(1, 35)} = 2.26$ $P = 0.10$
Melon-aphid	20	4.5 (4–5)	11.73	9.72	1.23	0.58	
<b>Adult period</b>							
Cotton-aphid	17	9 (7–11)	0	8.27	1.49	0	$\chi^2_{(1, 35)} = 30.64$ $P < 0.0001$
Melon-aphid	20	16 (13–18)	18	9.72	1.49	0.9	
<b>Pigweed</b>							
<b>Nymphal period</b>							
Cotton-aphid	15	6 (5–7)	8.93	7.5	1.22	0.59	$\chi^2_{(1, 28)} = 1.37$ $P = 0.24$
Melon-aphid	15	5 (5–6)	6.06	7.5	1.22	0.4	
<b>Adult period</b>							
Cotton-aphid	15	14 (12–15)	11	7.5	1.39	0.73	$\chi^2_{(1, 28)} = 6.31$ $P = 0.012$
Melon-aphid	15	13 (11–14)	4	7.5	1.39	0.26	
<b>Morning glory</b>							
<b>Nymphal period</b>							
Cotton-aphid	16	5 (4–6)	1.4	7.75	1.25	0.08	$\chi^2_{(1, 31)} = 25.54$ $P < 0.0001$
Melon-aphid	17	7 (6–7)	14.6	8.24	1.25	0.85	
<b>Adult period</b>							
Cotton-aphid	16	16 (14–17)	10.5	7.75	1.26	0.65	$\chi^2_{(1, 31)} = 4.67$ $P = 0.03$
Melon-aphid	17	14 (11–16)	5.5	8.24	1.26	0.32	

\*Cotton aphid – cotton associated population (CAP); melon aphid – melon associated population (MAP).

aphid: 4 days; melon aphid: 4.5 days). However, the HAPs responded differently to one of the two weed species, morning glory. The nymphal period of cotton-aphids reared on morning glory (5 days) was shorter than the nymphal period of melon-aphids reared on the same host (7 days).

*Adult period*

Irrespective of rearing hosts, the adult periods for two aphid populations varied significantly (CAP:  $\chi^2_{(1, 121)} = 83.28$ ,  $P < 0.0001$ ; MAP:  $\chi^2_{(1, 112)} = 91.73$ ,  $P < 0.0001$ ). Adult periods of both populations also varied with host plants, except for

watermelon. Cotton-aphids reared on cotton had the longest (21 days) and those reared on cucumber had the shortest (9 days) adult periods. Similarly, melon-aphids had the longest adult period on squash (19.5 days) and the shortest on cotton and okra (9 days each).

*Total fecundity*

Fecundity was affected by host association of populations ( $F_{(1, 223)} = 96.41$ ,  $P \leq 0.0001$ ). Irrespective of aphid population, host plants significantly affected aphid fecundity ( $F_{(6, 223)} = 695.24$ ,  $P \leq 0.0001$ ). Interactions between host plants and

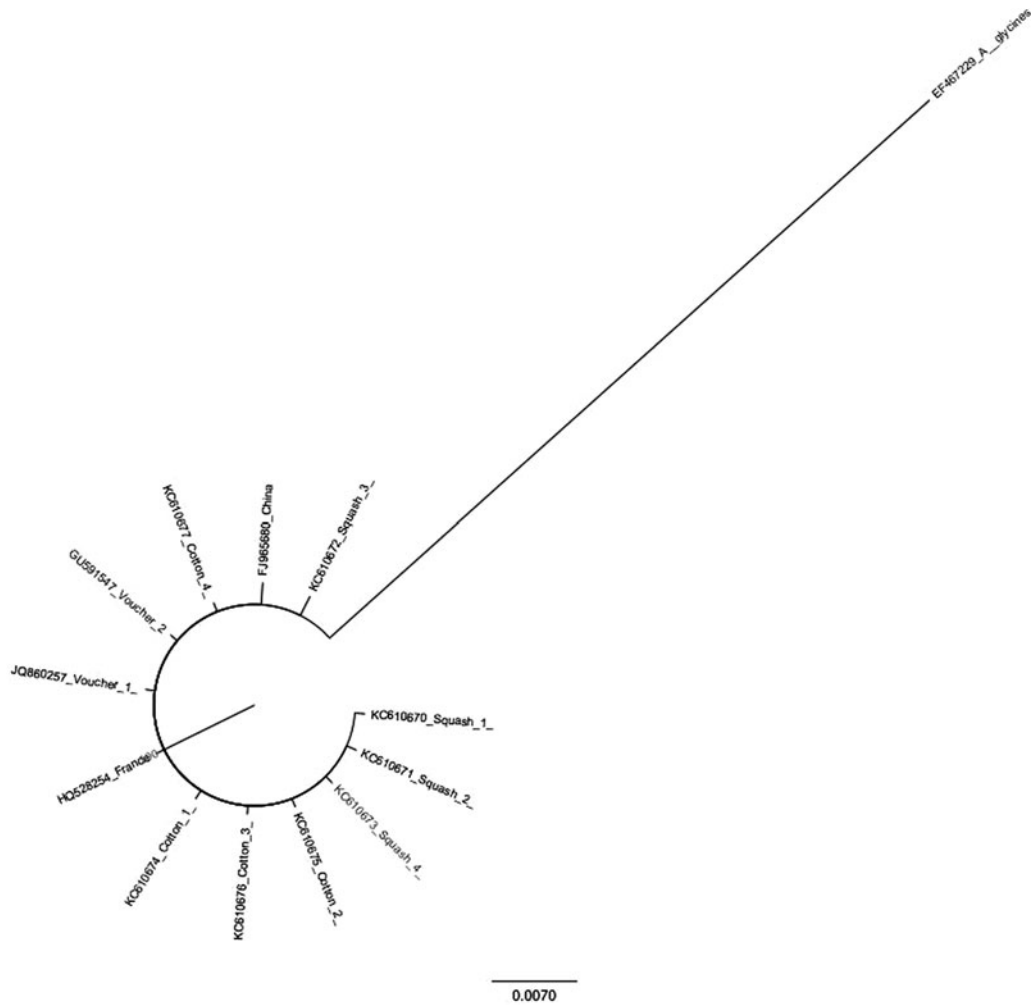


Fig. 1. Bayesian tree based on mitochondrial COI sequences of two host-associated *A. gossypii* populations and selected COI sequences of *Aphis gossypii* from GenBank.

HAPs also significantly affected fecundity ( $F_{(6, 223)} = 162.52$ ,  $P \leq 0.0001$ ). Fecundity of both melon and cotton aphids was greater on squash and watermelon than on cotton and okra (Fig. 2). However, fecundity of melon-aphids was reduced on cotton and okra than on squash, melon, cucumber, pigweed, and morning glory.

**Intrinsic rate of increase.** Host association in a population by itself did not have any effect on intrinsic rate of increase ( $F_{(1, 231)} = 3.52$ ,  $P = 0.062$ ). However, host plants influenced the intrinsic rate of increase for both aphid populations ( $F_{(6, 231)} = 59.58$ ,  $P \leq 0.0001$ ). Significant interactions between aphid populations and host-plant interactions were also observed ( $F_{(6, 231)} = 138.55$ ,  $P \leq 0.0001$ ). Intrinsic rate was reduced when individuals were exposed to their respective non-natal hosts. For example, when melon-aphids were reared on cotton or okra, their intrinsic rates of increase were less than when reared on hosts in the natal family, cucumber, squash, and melon (Fig. 3). Cotton aphids showed a similar trend in which their intrinsic rates of increase were higher on hosts in their natal family, cotton and okra, than on non-natal hosts such as squash and melon.

#### Phenotypical variation in *A. gossypii* populations

Regardless of host plants, melon-aphids had longer body length, hind tibia length, cornicle length and greater head capsule width than cotton-aphids (Fig. 4a–d). Furthermore, each of these phenotypical traits varied with host plant (Table 2). Significant interactions between HAPs and host plants were also observed for each phenotypical trait studied (Table 2). For instance, the melon aphids reared on squash and watermelon had greater body length, hind tibia length, cornicle length, and head capsule width than other host plants (Fig. 4a–d). The phenotypical characters of cotton aphids were in general smaller on cucumber than on most other hosts (Fig. 4a–d).

#### Discussion

We evaluated host utilization of two HAPs of *A. gossypii* using an array of aphid fitness and phenotypical traits. Our results demonstrate that these HAPs show plasticity in utilizing several hosts, thereby indicating absence of strict host

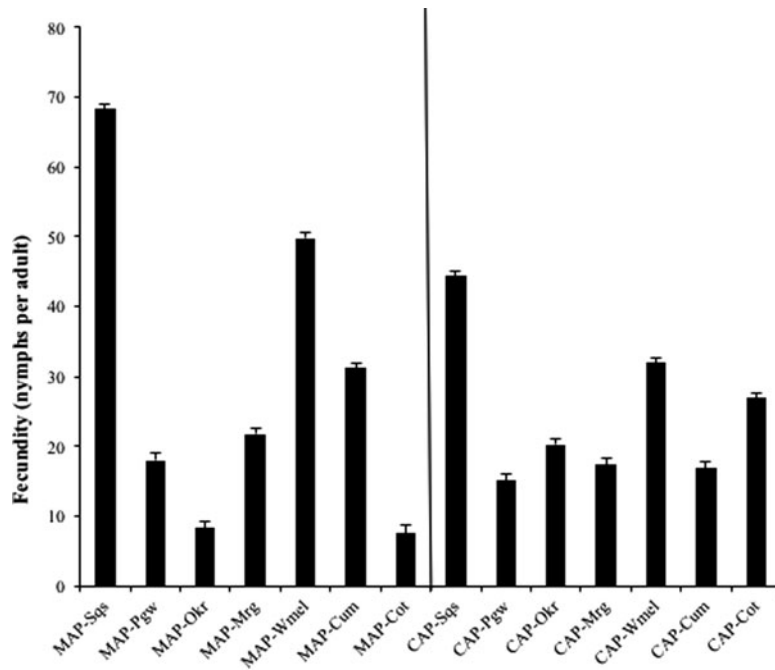


Fig. 2. Mean  $\pm$  SE values of fecundity in two host-associated *A. gossypii* populations as influenced by host plants. MAP- melon associated population, CAP- cotton associated population, Sqs- squash, Pgw- pigweed, Okr- okra, Mrg- morning glory, Wmel- watermelon, Cum- cucumber, and Cot- cotton.

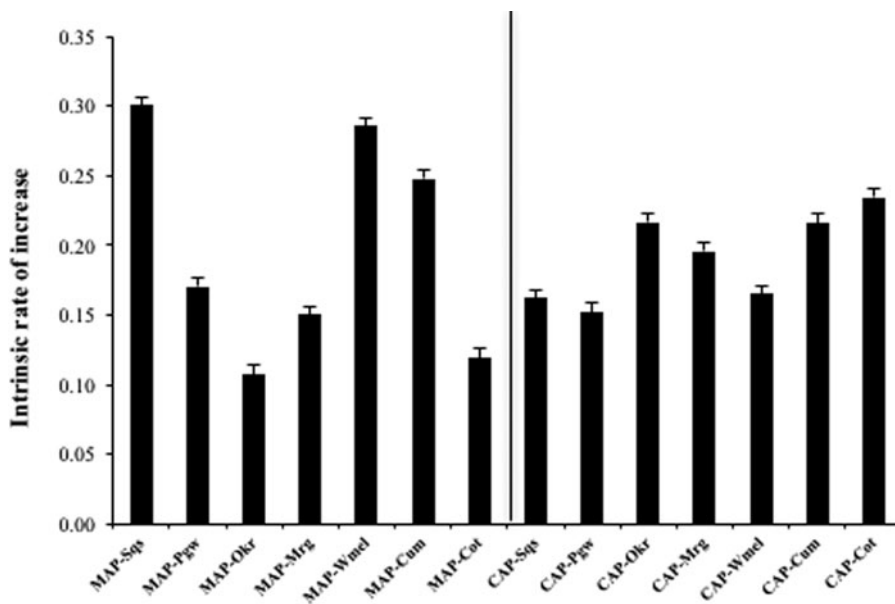


Fig. 3. Mean  $\pm$  SE values of intrinsic rate of increase in two host-associated *A. gossypii* populations as influenced by host plants. MAP- melon associated population, CAP- cotton associated population, Sqs- squash, Pgw- pigweed, Okr- okra, Mrg- morning glory, Wmel- watermelon, Cum- cucumber, and Cot- cotton.

specialization. Mitochondrial COI sequences from both of the aphid populations were identical, and did not reveal any evidence of genetic divergences between the two aphid populations. Mitochondrial genes often are used to detect recent divergence among taxa because of their faster rate of evolution than nuclear genes (Miyata *et al.*, 1982; Simon *et al.*, 1994; Graur

& Li, 1999; Wang *et al.*, 2013; Song *et al.*, 2016). Despite their increased rate of evolution, COI sequences often provide relatively low phylogenetic resolution at the population level, which consequently limits their use in validating lineages or biotypes. Further investigation of HAPs using higher resolution microsatellite markers, as shown by Brévault *et al.*

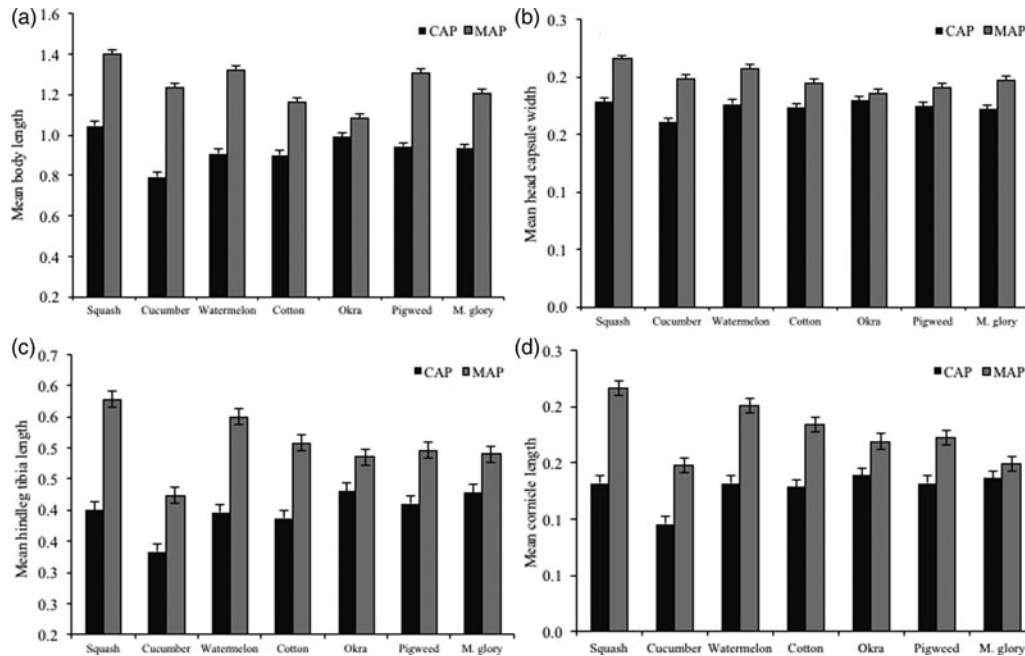


Fig. 4. Measurements (mean  $\pm$  SE) of four phenotypical traits in *A. gossypii* populations as influenced by host plants. Parameters illustrated in figures are as follows: (a) body length, (b) head capsule width, (c) hind tibia length, and (d) cornicle length.

Table 2. Influence of host associated populations (HAPs) and host plants on phenotypical parameters of *A. gossypii*.

Phenotypical parameters	Effects	df	F	P-value
Body length	HAP	1, 406	990.22	<0.0001
	Host	6, 406	28.56	<0.0001
	HAP $\times$ Host	6, 406	19.76	<0.0001
Distance between eyes	HAP	1, 406	206.72	<0.0001
	Host	6, 406	7.04	<0.0001
	HAP $\times$ Host	6, 406	6.46	<0.0001
Hind tibia length	HAP	1, 406	310.66	<0.0001
	Host	6, 406	17.26	<0.0031
	HAP $\times$ Host	6, 406	8.35	<0.0001
Cornicle length	HAP	1, 406	248.58	<0.0001
	Host	6, 406	14.64	<0.0001
	HAP $\times$ Host	6, 406	8.50	<0.0001

(2008), would have been more helpful to reveal genetic variability between cotton vs. squash-collected populations, if indeed there are differences. However, time and resource limitations precluded us from using higher resolution tools for this study. Apart from genetic divergence, other factors such as host range in the landscape and secondary endosymbiont composition could also influence aphids' plasticity in host utilization (Fukatsu *et al.*, 2001; Medina *et al.*, 2011; Brady & White, 2013).

Two HAPs of *A. gossypii* showed improved overall development on their natal hosts and other closely related hosts within the same plant families. For example, the nymphal development time of both HAPs was shorter on their natal hosts and hosts within that same family than on hosts in the non-natal family. Also, the adult longevity was greater on their

natal hosts and hosts within that family than on hosts in the non-natal family. Interestingly, the two HAPs performed intermediately on the two weed species (pigweed and morning glory), which suggests that in the absence of preferred crop hosts, aphids might be able to utilize alternate hosts. These results also indicate that these two HAPs are not host-specific biotypes and still retain their polyphagous abilities. In addition to the inter-population differences, there was substantial intra-population variation in both biological and phenotypic traits of the aphids utilizing different hosts within the natal host family. For example, melon-aphids utilizing squash lived significantly longer, produced more offspring, and were larger in size compared with melon-aphids utilizing watermelon and cucumber plants. Differential performance of *A. gossypii* on host plants within the same family can be attributed to variation in plant nutrient status and secondary metabolite profiles. Similar results were reported in the case of *Uroleucon* aphid while utilizing different *Solidago* hosts (Asteraceae), where variation in host nutritional quality influenced the performance of aphids (Moran, 1981).

Although the two HAPs of *A. gossypii* in our study showed distinct patterns with respect to several biological and phenotypic traits following exposure to host plants in both natal and non-natal families, this does not necessarily warrant calling these two HAPs as host specialized populations or biotypes. This is especially true based on fecundity, which is a good measure of aphids' performance on a particular host (Lamb *et al.*, 2009). In our study, we observed distinct trends for two HAPs. We found that the fecundity of melon-aphids was reduced by 89% on the non-natal host (cotton), while the fecundity of cotton-aphids rather increased by 66% on their non-natal host, squash. Thus, it appears that the melon-aphids are more specialized on cucurbit hosts (melon and squash) and less amenable to alternate host plants in non-natal families. On

the contrary, the cotton-aphids did not appear to specialize on their natal hosts (Malvaceae); rather, they exhibited better reproductive performance on non-natal hosts such as watermelon and squash. An earlier study by Chen *et al.* (1999) also reported similar results, in which melon aphids performed better on watermelon than cotton aphids. This enhanced performance was attributed to increased tolerance of melon aphids to pyrazole, an anti-herbivory secondary phloem metabolite. Thus, secondary metabolites, apart from nutrients such as amino acids, may have contributed to the differential responses of aphids to natal and non-natal hosts thereby affecting aphid growth and fitness.

Phenotypical measurements such as body length, tibial length, and cornicle length have been used to describe variation in aphid populations utilizing different host plants (Margaritopoulos *et al.*, 2000; Lee & Lee, 2013). Such phenotypical variations in populations experiencing different feeding environments may directly affect aphid fitness (Honek, 1993; Via & Shaw, 1996; de Kogel *et al.*, 1999; Pereira & de Paula, 2009). Melon-aphids were significantly larger than cotton-aphids regardless of developmental host plants. The inherent size difference in two HAPs might be due to the quality of original feeding hosts. This may have led to the larger body size in melon-aphids along with their higher reproductive performance compared with aphids that developed on cotton. Similar observations were made by Wool & Hales (1997) in Australian *A. gossypii* populations, which were larger in size upon rearing on cucumber and rockmelon compared with aphid populations reared on cotton, broad bean, and eggplant. Relationship of host plant quality and aphid's reproductive performance was also validated in pea aphids, *Acyrtosiphon pisum* (Harris), which produced more offspring on *Vicia faba* L., a supposedly superior host than *Lathyrus pratensis* L. (McLean *et al.*, 2009). Interestingly, this host effect on *A. gossypii* is observable within a generation. In this study, when larger sized melon-aphids were exposed to either cotton or okra plants as developmental hosts, a reduction in size was observed. Similar trends were observed in the case of two other phenotypical traits evaluated, hind tibia and cornicle length.

Plant quality and secondary metabolite profiles play a significant role in aphid's performance and phenotypic development (Moran, 1981; Douglas, 1993; Awmack & Leather, 2002; Powell *et al.*, 2006). In addition to these plant attributes, the maternal effect –progeny's performance as a result of the mother's experience is a critical determinant of aphid fitness (Mousseau & Dingle, 1991; Fox *et al.*, 1995). A strong maternal effect on the subsequent offspring generations could be expected as aphids have overlapping telescopic generations (Dixon, 1998). Several studies have examined as to whether the maternal effect on subsequent offspring performance plays a significant role (Via, 1991; Zehnder & Hunter, 2007; McLean *et al.*, 2009; Tariq *et al.*, 2010). Aphids may need to be followed for several generations in the novel environment in order to eliminate any bias associated with the presence of maternal effects (Mousseau & Dingle, 1991; Olivares-Donoso *et al.*, 2007). To separate maternal effects from host effects in *A. gossypii* populations, future studies could investigate how long (number of generations) the mother has to be on a specific non-natal host in order to observe the maternal effect on its offspring.

Our study further provides insights into the potential role of annual weed species as alternate hosts on the biology of *A. gossypii* in the context of agricultural landscape. Among the

several dominant weed species in farmscapes of the south-eastern United States, Pigweed, and morning glory are prominent (Webster & MacDonald, 2001). In a given year, the availability of cultivated crop may vary temporally and spatially within these farmscapes. Also, several other factors such as deteriorating quality of senescing crops, overcrowding of aphid populations, and pesticide applications may drive individuals to seek alternate hosts, which could be one or more annual weed species. This study clearly shows that regardless of natal hosts, *A. gossypii* can sub-optimally exploit these two annual weed species, pigweed, and morning glory. While there are several reports of *A. gossypii* using perennial plants such as *Hibiscus* for overwintering purposes (Liu *et al.*, 2008a, b; Razmjou *et al.*, 2010), records are limited when it comes to annual non-crops serving as alternate hosts (Perng, 2002; Margaritopoulos *et al.*, 2009). In Taiwan, Perng (2002) documented that among the four weed species, *Solanum nigrum* L. (black nightshade) was a better host of *A. gossypii* than *Ageratum houstonium* Mill., *Bidens pilosa* L., and *Spermacoce latifolia* (Aubl.). Not all these species may be equally important for every geographical region. Nevertheless, regionally dominant weed hosts could provide important information and aid in understanding the dynamics of aphid populations in an agricultural landscape.

### Concluding remarks

We observed that the two HAPs of *A. gossypii* in this study exhibited plasticity in host utilization. The HAPs were not necessarily specialized or genetically diverged. The lack of severe fitness costs on non-natal hosts suggests that divergence into host-specific biotypes is unlikely. Despite this, identifying the common patterns associated with host plant utilization by multiple HAPs in a farmscape/landscape setting could potentially be helpful in understanding dispersal patterns and in designing the pest management strategies. If such HAPs evolve in to specific biotypes, then pest management strategies could be tailored for each biotype. An example being the case of whitefly biotypes B and Q, wherein the Q biotype has increased capabilities to develop resistance to insecticides than B, and typically B is more competitive than Q (Horowitz & Ishaaya, 2014). Timely knowledge on host specialized populations/lineages/biotypes and understanding their dispersal patterns would serve as valuable information from a commercial agriculture standpoint.

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### References

- Agarwala, B.K. & Choudhury, P.R. (2013) Host races of the cotton aphid, *Aphis gossypii*, in asexual populations from wild plants of taro and brinjal. *Journal of Insect Science* **13**, 1–13.
- Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology* **47**, 817–844.
- Bancroft, J.S. (2006) Comparison of two species of aphids (Hemiptera: Aphididae) on nine host plants using age



- specific fecundity and survival. *Southwestern Entomologist* **31**, 233–243.
- Barman, A.K., Parajulee, M.N., Sansone, C.G., Suh, C.P.C. & Medina, R.F.** (2012) Geographic pattern of host-associated differentiation in the cotton fleahopper, *Pseudatomoscelis seriatius*. *Entomologia Experimentalis et Applicata* **143**, 31–41.
- Bernays, E.A. & Chapman, R.F.** (1994) *Host-Plant Selection by Phytophagous Insects*. New York, Chapman and Hall.
- Blackman, R.L. & Eastop, V.F.** (2000) *Aphids on the World's Crops. An Identification and Information Guide*. 2nd edn. New York, Wiley.
- Brady, C.M. & White, J.A.** (2013) Cowpea aphid (*Aphis craccivora*) associated with different host plants has different facultative endosymbionts. *Ecological Entomology* **38**, 433–437.
- Brévault, T., Carletto, J., Linderme, D., & Vanlerberghe-Masutti, F.** (2008) Genetic diversity of the cotton aphid *Aphis gossypii* in the unstable environment of a cotton growing area. *Agricultural and Forest Entomology* **10**, 215–223.
- Carletto, J., Lombaert, E., Chavigny, P., Brevault, T., Lapchin, L. & Vanlerberghe-Masutti, F.** (2009) Ecological specialization of the aphid *Aphis gossypii* glover on cultivated host plants. *Molecular Ecology* **18**, 2198–2212.
- Chen, J.Q., Rahbe, Y., Delobel, B.** (1999) Effect of pyrazole compounds from melon on the melon aphid *Aphis gossypii*. *Phytochemistry* **50**, 1117–1122.
- de Kogel, W.J., Bosco, D., van der Hoek, M. & Mollema, C.** (1999) Effect of host plant on body size of *Frankliniella occidentalis* (Thysanoptera : Thripidae) and its correlation with reproductive capacity. *European Journal of Entomology* **96**, 365–368.
- Dixon, A.F.G.** (1998) *Aphid Ecology: An Optimization Approach*. 2nd edn. London, Chapman & Hall.
- Douglas, A.E.** (1993) The nutritional quality of phloem sap utilized by natural aphid populations. *Ecological Entomology* **18**, 31–38.
- Ebert, T.A. & Cartwright, B.** (1997) Biology and ecology of *Aphis gossypii* glover (Homoptera: Aphididae). *Southwestern Entomologist* **22**, 116–153.
- Fox, L.R. & Morrow, P.A.** (1981) Specialization: species property or local phenomenon? *Science* **211**, 887–893.
- Fox, C.W., Waddell, K.J. & Mousseau, T.A.** (1995) Parental host-plant affects offspring life-histories in a seed beetle. *Ecology* **76**, 402–411.
- Fukatsu, T., Tsuchida, T., Nikoh, N. & Koga, R.** (2001) *Spiroplasma* symbiont of the pea aphid, *Acyrtosiphon pisum* (Insecta: Homoptera). *Applied and Environmental Microbiology* **67**, 1284–1291.
- Funk, D.J. & Bernays, E.A.** (2001) Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae* aphids. *Ecology* **82**, 726–739.
- Futuyma, D.J.** (1976) Food plant specialization and environmental predictability in lepidoptera. *American Naturalist* **110**, 285–292.
- Futuyma, D.J. & Moreno, G.** (1988) The evolution of ecological specialization. *Annual Review of Ecology and Systematics* **19**, 207–233.
- Gao, X. & Liu, X.D.** (2008) Differentiation of cotton and cucumber specialized aphids of *Aphis gossypii* glover in capacity to produce sexuales. *Acta Entomologica Sinica* **51**, 40–45.
- Gotthard, K., Margraf, N. & Rahier, M.** (2004) Geographic variation in oviposition choice of a leaf beetle: the relationship between host plant ranking, specificity, and motivation. *Entomologia Experimentalis et Applicata* **110**, 217–224.
- Graur, D. & Li, W.H.** (1999) *Fundamentals of Molecular Evolution*. Sunderland, MA, Sinauer Associates.
- Gulderson, J.A., Tigges, W.T. & Devrijer, P.W.F.** (1994) Host races of *Aphis gossypii* (Homoptera: Aphididae) on cucumber and chrysanthemum. *Environmental Entomology* **23**, 1235–1240.
- Hall, B.G.** (2001) *Phylogenetic Trees Made Easy*. Sunderland, MA, Sinauer Associates.
- Honek, A.** (1993) Intraspecific variation in body size and fecundity in insects- a general relationship. *Oikos* **66**, 483–492.
- Horowitz, A.R. & Ishaaya, I.** (2014) Dynamics of biotypes B and Q of the whitefly *Bemisia tabaci* and its impact on insecticide resistance. *Pest Management Science* **70**, 1568–1572.
- Jaenike, J.** (1990) Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics* **21**, 243–273.
- Lamb, R.J., MacKay, P.A. & Migui, S.M.** (2009) Measuring the performance of aphids: fecundity versus biomass. *Canadian Entomologist* **141**, 401–405.
- Lee, W. & Lee, S.** (2013) Molecular and morphological characterization of two aphid genera, *Acyrtosiphon* and *Aulacorthum* (Hemiptera: Aphididae). *Journal of Asia-Pacific Entomology* **16**, 29–35.
- Liu, X.D., Zhai, B.P. & Zhang, X.X.** (2008a) Specialized host-plant performance of the cotton aphid is altered by experience. *Ecological Research* **23**, 919–925.
- Liu, X.D., Zhai, B.P., Zhang, X.X. & Gu, H.N.** (2008b) Variability and genetic basis for migratory behaviour in a spring population of the aphid, *Aphis gossypii* glover in the Yangtze river valley of China. *Bulletin of Entomological Research* **98**, 491–497.
- Margaritopoulos, J.T., Tsitsipis, J.A., Zintzaras, E. & Blackman, R.L.** (2000) Host-correlated morphological variation of *Myzus persicae* (Hemiptera : Aphididae) populations in Greece. *Bulletin of Entomological Research* **90**, 233–244.
- Margaritopoulos, J.T., Tzortzi, M., Zarpas, K.D. & Tsitsipis, J.A.** (2009) Predominance of parthenogenetic reproduction in *Aphis gossypii* populations on summer crops and weeds in Greece. *Bulletin of Insectology* **62**, 15–20.
- McBride, C.S.** (2007) Rapid evolution of smell and taste receptor genes during host specialization in *Drosophila sechellia*. *Proceedings of National Academy Sciences USA* **104**, 4996–5001.
- McLean, A.H.C., Ferrari, J. & Godfray, H.C.J.** (2009) Effects of the maternal and pre-adult host plant on adult performance and preference in the pea aphid, *Acyrtosiphon pisum*. *Ecological Entomology* **34**, 330–338.
- Medina, R.F., Nachappa, P. & Tamborindeguy, C.** (2011) Differences in bacterial diversity of host-associated populations of *Phylloxera notabilis* Pergande (Hemiptera: Phylloxeridae) in pecan and water hickory. *Journal of Evolutionary Biology* **24**, 761–771.
- Miyata, T., Hayashida, H., Kikuno, R., Hasegawa, M., Kobayashi, M. & Koike, K.** (1982) Molecular clock of silent substitution: at least six-fold preponderance of silent changes in mitochondrial genes over those in nuclear genes. *Journal of Molecular Evolution* **19**, 28–35.
- Moran, N.** (1981) Intraspecific variability in herbivore performance and host quality- A field-study of *Uroleucon caligatus* (Homoptera, Aphididae) and its *Solidago* hosts (Asteraceae). *Ecological Entomology* **6**, 301–306.
- Mousseau, T.A. & Dingle, H.** (1991) Maternal effects in insect life histories. *Annual Review of Entomology* **36**, 511–534.
- Najar-Rodriguez, A.J., McGraw, E.A., Hull, C.D., Mensah, R.K. & Walter, G.H.** (2009) The ecological differentiation of asexual lineages of cotton aphids: alate behaviour, sensory physiology, and differential host associations. *Biological Journal of the Linnean Society* **97**, 503–519.
- Olivares-Donoso, R., Troncoso, A.J., Tapia, D.H., Aguilera-Olivares, D. & Niemeyer, H.M.** (2007) Contrasting

- performances of generalist and specialist *Myzus persicae* (Hemiptera : Aphididae) reveal differential prevalence of maternal effects after host transfer. *Bulletin of Entomological Research* **97**, 61–67.
- Pereira, C.D. & de Paula, C.L.** (2009) Plasticity on size and fecundity of the aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) utilizing distinct hosts. *Bioscience Journal*, **25**, 122–130.
- Perng, J.J.** (2002) Life history traits of *Aphis gossypii* glover (Hom., Aphididae) reared on four widely distributed weeds. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie* **126**, 97–100.
- Powell, G., Tosh, C.R. & Hardie, J.** (2006). Host plant selection by aphids: behavioral, evolutionary, and applied perspectives. *Annual Review of Entomology* **51**, 309–330.
- Razmjou, J., Vorburger, C., Moharramipour, S., Mirhoseini, S.Z. & Fathipour, Y.** (2010) Host-associated differentiation and evidence for sexual reproduction in Iranian populations of the cotton aphid, *Aphis gossypii*. *Entomologia Experimentalis et Applicata* **134**, 191–199.
- Ronquist, F. & Huelsenbeck, J.P.** (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.
- Satar, S., Kersting, U. & Yokomi, R.** (2013) Presence of two host races of *Aphis gossypii* glover (Hemiptera: Aphididae) collected in Turkey. *Annals of Applied Biology* **162**, 41–49.
- Schoonhoven, L.M., van Loon, J.J.A. & Dicke, M.** (2005) *Insect-Plant Biology*. 2nd edn. Cambridge, University Press.
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P.** (1994) Evolution, weighing, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America* **87**, 651–701.
- Singer, M.C. & McBride, C.S.** (2012) Geographic mosaics of species' association: a definition and an example driven by plant-insect phenological synchrony. *Ecology* **93**, 2658–2673.
- Song, N., Zhang, H., Li, H. & Cai, W.** (2016) All 37 mitochondrial genes of aphid *Aphis craccivora* obtained from transcriptome sequencing: implications for the evolution of aphids. *PLoS ONE* **11**, e0157857. doi: 10.1371/journal.pone.0157857.
- Stotz, G.C., Suárez, L.H., Gonzáles, W.L. & Gianoli, E.** (2013) Local host adaptation and use of a novel host in the seed beetle *Megacerus eulophus*. *PLoS ONE* **8**, e53892.
- Sword, G.A., Joern, A., & Senior, L.B.** (2005) Host plant-associated genetic differentiation in the snakeweed grasshopper, *Hesperotettix viridis* (Orthoptera : Acrididae). *Molecular Ecology* **14**, 2197–2205.
- Tariq, M., Wright, D.J. & Staley, J.T.** (2010) Maternal host plant effects on aphid performance: contrasts between a generalist and a specialist species on Brussels sprout cultivars. *Agricultural and Forest Entomology* **12**, 107–112.
- Thompson, J.N.** (2005) *The Geographic Mosaic of Coevolution*. Chicago, University of Chicago Press.
- Via, S.** (1991) Specialized host plant performance of pea aphid clones is not altered by experience. *Ecology* **72**, 1420–1427.
- Via, S. & Shaw, A.J.** (1996) Short-term evolution in the size and shape of pea aphids. *Evolution* **50**, 163–173.
- Wang, Y., Huang, X.L., Qiao, G.X.** (2013) Comparative analysis of mitochondrial genomes of five aphid species (Hemiptera: Aphididae) and phylogenetic implications. *PLoS ONE* **8**, e77511. doi: 10.1371/journal.pone.0077511.
- Webster, T.M. & MacDonald, G.E.** (2001) A survey of weeds in various crops in Georgia. *Weed Technology* **15**, 771–790.
- Wool, D. & Hales, D.F.** (1997) Phenotypic plasticity in Australian cotton aphid (Homoptera: Aphididae): host plant effects on morphological variation. *Annals of the Entomological Society of America* **90**, 316–328.
- Wyatt, I.J. & White, P.F.** (1977) Simple estimation of intrinsic increase rates for aphids and tetranychid mites. *Journal of Applied Ecology*, **14**, 757–766.
- Zehnder, C.B. & Hunter, M.D.** (2007) A comparison of maternal effects and current environment on vital rates of *Aphis nerii*, the milkweed-oleander aphid. *Ecological Entomology* **32**, 172–180.