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ORIGINAL ARTICLE

Comparative Biological and Molecular studies on host-adapted populations of the melon aphid, *Aphis gossypii* (Hemiptera: Aphididae) in India

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ABSTRACT
The melon aphid, Aphis gossypii Glover (Hemiptera: Aphididae) is a cosmopolitan, polyphagous aphid species. It exhibits host-associated genetic structure that results in evolution of biotypes and cryptic species favoring host adaptation and reproductive isolation. The relationship between host preference and genetic variation is not clear in aphids, especially A. gossypii. Studies were conducted to determine if host-adapted races of A. gossypii are indeed present in multiple asexual lineages associated with the plants belonging to Malvaceae, Cucurbitaceae and Solanaceae in India based on biological attributes and analysis of partial mitochondrial cytochrome oxidase subunit gene I (mtCOI) and tRNA-leucine +cytochrome oxidase subunit gene II (tRNA/COII). Results indicated significant variations in biological performance of A. gossypii on various host plants. Cucumber adapted aphid clones seemed to be the most successful while they were highly prolific coupled with more longevity when compared to other host plants. Comparison of partial COI and tRNA/COII sequences showed that there are few variable sites accounting to a total variation of 0.19% and 0.17% respectively. Further, the phylogram obtained indicated that intra-specific variation between them was extremely low and thus no host specificity was evident in Indian populations of A. gossypii. Haplotype network analysis based on COI and tRNA/COII sequences revealed that A. gossypii haplotypes obtained were not associated with host plant families in India and the ancestral haplotype is widely distributed and could successfully thrive on host plants irrespective of three plant families mentioned above. These results unambiguously proved that A. gossypii is a single cosmopolitan species with no major host associated genetic differences in India. However, the variation in biological attributes between aphid clones on various hosts raised doubts about the genetic homogeneity of A. gossypii. The differences in biology and behaviour observed on various hosts could be attributed to physiological variations, differences in host plant nutrient composition, endosymbionts and to unknown co-existing biotic factors.
Keywords; *Aphid, biological performance, COI, host specificity, tRNA/COII*

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INTRODUCTION

The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is one of the most important cosmopolitan and extremely polyphagous aphid species capable of causing direct and indirect damage to various crops worldwide [1]. It reproduces primarily by parthenogenesis on various host plants in warmer parts of the world [1] and cyclical parthenogenesis with sexually-produced eggs in North America [2], Japan [3] and China [4] involving an alternation of hosts between a winter primary host and spring summer secondary host/s [5]. Host plants attacked include cotton, okra and *Hibiscus* (Malvaceae); pumpkin, cucumber, zucchini and watermelon (Cucurbitaceae); and aubergine, chili pepper and tomato

(Solanaceae). It exhibits host-associated genetic structure and is known to transmit >75 plant viruses [6]. It is the most biologically diverse species of aphids; a polyphagous species in a family where most are host specialists [7]. Host specificity in *A. gossypii* influences reproductive isolation when migration occurs from one host to other due to pre-mating or post-mating selection against migrants and hybrid progeny [8, 9]. This results in evolution of biotypes and cryptic species favouring reproductive isolation, which is evident in the greenbug aphid, *Schizaphis graminum* (Rondani) [10], the pea aphid, *Acyrthosiphon pisum* (Harris) [11], grain aphid, *Sitobion avenae* (Fabricius) [12], yellow clover aphid, *Therioaphis trifolii* (Monell) [13] and the black bean aphid, *Aphis fabae* Scopoli [14]. The detection of differentiated host races within apparently polyphagous aphid species suggests an ongoing process of speciation by adaptation to distinct host plants [9].

The significant differences in biological performance of *A. gossypii* recorded on different hosts [15, 16] indicate the presence of host-adapted genotypes/host races. In light of this, it is important to establish whether *A. gossypii* lineages exhibiting different host associations are diverging and becoming distinct taxonomic entities in India. If this is so then they show consistent differences in their biology and genetics. Therefore the present study was conducted to investigate the biological variations in terms of developmental and reproductive fitness of host-adapted Indian populations of *A. gossypii* on 6 host plant species from Malvaceae, Cucurbitaceae and Solanaceae corroborated by mitochondrial DNA analyses. Since, host plant switching by aphids is attributed to selection of a variant parthenogenetic embryo(s) developing in the aphid feeding on a new host [17], the performance of clones of *A. gossypii* originating from the plant families Malvaceae, Cucurbitaceae and Solanaceae were subjected to reciprocal host transfers. The colonization success was recorded to test the ability of individual aphid to survive and reproduce in a new host environment [18].

Earlier reports based on molecular markers such as microsatellites, mitochondrial cytochrome b gene and elongation factor (EF) 1-α suggested that *A. gossypii* exhibits host-associated genetic structure [19, 20, 21, 22]. However, several reports based on microsatellites, Random Amplified Polymorphic DNA (RAPD), Internal Transcribed Spacer2 (ITS2) rDNA, mtCOI, tRNA/mtCOII and EF 1-α markers suggested that *A. gossypii* do not possess host-associated genetic variability [23, 24, 25, 26, 27]. Thus the taxonomic status of host-associated forms of *A. gossypii* is complex and there is a lack of comprehensive knowledge on the genetic structure. Reports that discuss host specialization in Indian populations of *A. gossypii* based on their biological performance coupled with mitochondrial DNA analyses is a need of the hour to gain insights into ecological speciation and to devise effective management tactics against this notorious pest.

Molecular tools have proved (often if not always) efficient in resolving taxonomic status of aphids [7, 28, 29,]. Various molecular markers have been employed for deciphering host associated genetic differences in aphids: mitochondrial cytochrome oxidase - mtCOI and/or mtCOII [25, 30], RAPD [31], microsatellites [32, 33]. Since mitochondrial markers such as COI and tRNA/COII have maternal inherited characteristics, rapid rate of evolutionary change and reliable inter-specific variation [34] they have been employed in this study to resolve the genetic structure of *A. gossypii.* The present study was aimed to obtain some preliminary information on host association among various populations of *A. gossypii* in India. The adoption of new aphid hosts might have required some specialization that would be reflected by using DNA sequences. Therefore, mitochondrial cytochrome oxidase subunit gene I (COI) "barcode" (658 bp) and tRNA-leucine + cytochrome *c* oxidase subunit gene II (tRNA/COII) (813 bp) nucleotide sequences of *A. gossypii* obtained from aphid clones used for biological experiments [35] were used for diversity analysis in comparison with other available Indian sequences in the National Center for Biotechnology Information (NCBI), GenBank. The prime objectives of this study were to measure biological differences between populations of *A. gossypii* on a range of host plant species, to assess the effect of transferring aphid clones from their host-adapted line to another host, and to use mitochondrial DNA analyses to judge whether there were any host-associated genetic differences present in the Indian *A. gossypii* populations.

MATERIALS AND METHODS

Maintenance of stock culture and morphological identification

The aphid samples utilized in this study were collected on 6 different plant species frequently infested by *A. gossypii*. They are cotton (*Gossypium hirsutum* L.; Malvaceae, hybrid), okra cv. Arka Anamika (*Abelmoschus esculentus* L.*,* Malvaceae), cucumber hyb. Malini (*Cucumis sativus* L.; Cucurbitaceae), watermelon cv. Arka Manik (*Citrullus lanatus* Thunb.), aubergine cv. Arka Anand (*Solanum melongena* L; Solanaceae) and chili pepper cv. Arka Meghana (*Capsicum frutescens* L.) cultivated at 6 different locations, separated by about 2000 m distance from each other, at the Indian Institute of Horticultural Research (IIHR), Bengaluru, India (12^0 58' N; 77^0 35'E) during 2011. The aphids from these crops were used to

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raise stock cultures. Live aphids along with the plant material were transferred to the laboratory, where a single apterous, parthenogenetic, viviparous, adult female was used to establish a stock culture in the laboratory and were maintained on respective hosts in the insectary. Specimens from each of the 6 populations used for morphological as well as molecular analyses were collected and preserved in 70% ethanol at -20°C until further use. Aphids were identified morphologically by Dr. Sunil Joshi of the National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India.

Developmental and reproductive performance of *A. gossypii*

Experiments were conducted as completely randomized designs. Twenty apterae (yellow forms) were selected randomly from each of the laboratory cultures and transferred onto an excised leaf of natal hosts in a Petri dish. After approximately 24 h, fifteen new-born aphids were randomly selected and kept on fifteen leaf discs (replications) to initiate a life table study on respective hosts. All replications in which nymphs died within 24 h after transfer were omitted. Leaves were excised from 10 to 12 week-old cotton, okra, cucumber, watermelon, aubergine and chili pepper plants. All leaves were rinsed using distilled water and leaf discs of approximately 8 cm diameter were excised using a razor blade except for watermelon and chili pepper where detached fresh leaf was kept on the wet cotton wool in the Petri dishes. Leaf discs were placed upside down on 1% agar bed in disposable polystyrene Petri dishes (85 mm×15 mm) and one first instar nymph (< 12 h old) selected randomly was introduced using a moistened brush onto each leaf disc and maintained at 27 ± 1°C, 70 ± 5% RH and a photoperiod of 12:12 h L:D.

Individual nymphs were checked every 12 h. Development and mortality was recorded until they reached adulthood and produced the first parthenogenetic offsprings. The presence of exuviae was used to determine molting time and all newborn nymphs were removed soon after the measurements were recorded. After the first parthenogenesis, reproduction of aphids on the leaf discs was recorded every 24 hours. Observations continued daily until death of all adults. New-born aphids were counted daily and transferred to other Petri dishes. Aphids were transferred to fresh leaf discs every 2 or 3 days to maintain the vigor of the aphid culture. As a result, developmental time from birth of a nymph to its final molt, generation time from the birth of a nymph to the onset of reproduction, reproductive duration from the birth of the first nymph to the last nymph and fecundity were recorded.

The intrinsic rate of increase (*r*) was determined by Euler equation, $\sum e^{-rx} l_x m_x = 1$, where x is the age in days (including immature stages), r is the intrinsic rate of increase, and l_x is the proportion of individuals alive at time x of an original cohort (including immature mortality). The first day of aphid's life was set as the first pivotal age with age divided into increments of one day. The variable m_x is the mean number of offspring produced per surviving aphid during the age interval x (1 d). The life table parameters: gross reproductive rate (GRR $=\sum m_x$), net reproductive rate ($R_o=\sum l_x m_x$), finite rate of increase ($\lambda=e^r$), mean generation time ($GT=\ln R_o/r$), and doubling time ($DT=\ln 2/r$) were calculated [36]. A widely applied Jackknife method [37, 38, 39, 40, 41] was used to estimate the SEM of the calculated life table statistics, including GRR, R_o, λ, GT and DT after computing the intrinsic rate of increase (r) for the original data (r_{all}). The jackknife pseudo-value r_j was calculated for the n samples using the equation $r_j = n * r_{all} - (n-1) * r_i$. Further, these values were subjected to one way analysis of variance (ANOVA) using PROC GLM of SAS V9.3 [42] in order to determine the biological variations of *A. gossypii* on 6 different host plant species. The significance was tested at 5% level of probability using Fischer protected Least Significant Difference (LSD) [43].

Host transfer experiments

Melon aphid clones (yellow apterous adults) on 6 different host plants of 3 plant families reared in the laboratory were subjected to reciprocal host transfers between families to record the colonization success in a new host environment. In the first experiment, *A. gossypii* was transferred individually from cotton and okra to cucumber, watermelon, aubergine and chili pepper. In the second, aphids from cucumber and watermelon were transferred individually onto cotton, okra, aubergine and chili pepper. In the third, *A. gossypii* was transferred individually from aubergine and chili pepper onto cotton, okra, cucumber and watermelon. Individual nymphs, 0–12 hr old, were released on fresh excised leaf discs as mentioned earlier. These aphids were allowed to settle and were observed to see if they survived and produced nymphs for the first generation. Twenty five replicates were used in each set to record the success rate of survival. Aphids that either failed to develop to the adult stage in the first generation or failed to produce second or third generation were considered to be unsuccessful [18].

Molecular studies

Sequence analysis

The differences in COI and tRNA/COII nucleotide sequences of *A. gossypii* obtained from aphid clones used for biological experiments [35] together with sequences of 16 Indian *A. gossypii* populations [25]

available in the NCBI were determined using the sequence alignment editor BioEdit v7.0.5.3 [44]. Details of COI and tRNA/COII sequences of *A. gossypii* retrieved from NCBI, GenBank along with their accession numbers are given in Table 1. All the sequences were aligned in BioEdit.v7.2.5 program using Clustal W algorithm. The alignment was further analyzed using MEGA v5.0 [45]. In addition to these sequences, COI and tRNA/COII sequences of *Aphis glycines* Matsumura (retrieved from NCBI) were selected as outgroup for the phylogenetic analyses, which included Bayesian inference (BI) and maximum likelihood (ML). Of an 813 bp region determined for tRNA/COII, a 702 bp region could be utilized for building the phylogenetic tree with sequence of *A. glycines* (outgroup) obtained from GenBank (COI: GQ904088 and tRNA/COII: GQ904150).

Table 1. Details of *Aphis gossypii* deposits from India retrieved from NCBI and its accession numbers

Sample No.	Host plant species	NCBI Accessions	
		COI	COII
1	Cotton[1]	KF446143	KF446161
2	Cotton[1]	KF446149	KF446167
3	Cotton[1]	KF446155	KF446173
4	Okra[1]	KF446144	KF446162
5	Okra[1]	KF446150	KF446168
6	Okra[1]	KF446156	KF446174
7	Cucumber[1]	KF446145	KF446163
8	Cucumber[1]	KF446151	KF446169
9	Cucumber[1]	KF446157	KF446175
10	Watermelon[1]	KF446146	KF446164
11	Watermelon[1]	KF446152	KF446170
12	Watermelon[1]	KF446158	KF446176
13	Aubergine[1]	KF446147	KF446165
14	Aubergine[1]	KF446153	KF446171
15	Aubergine[1]	KF446159	KF446177
16	Chili pepper[1]	KF446148	KF446166
17	Chili pepper[1]	KF446154	KF446172
18	Chili pepper[1]	KF446160	KF446178
19	Cotton[2]	JQ067099	JQ067113
20	Cotton[2]	JQ067101	JQ067115
21	Cotton[2]	JQ067107	JQ067121
22	Cotton[2]	JQ067108	JQ067122
23	Cotton[2]	JQ690329	JQ690299
24	Cotton[2]	JQ690330	JQ690300
25	Cotton[2]	JQ690331	JQ690301
26	Cotton[2]	JQ690335	JQ690305
27	Cotton[2]	JQ690336	JQ690306
28	Hibiscus[2]	JQ067106	JQ067120
29	Watermelon[2]	JQ067100	JQ067114
30	Watermelon[2]	JQ067102	JQ067116
31	Watermelon[2]	JQ067103	JQ067117
32	Watermelon[2]	JQ067104	JQ067118
33	Watermelon[2]	JQ067105	JQ067119
34	Sponge gourd[2]	JQ690332	JQ690302
35	Pumpkin[2]	JQ690334	JQ690304
36	Aubergine[2]	JQ690333	JQ690303

[1]Lokeshwari *et al*. (2014), [2]Rebijith *et al*. (2012)

Incongruence length difference (ILD) test was performed in PAUP v4b10 [46]. A Bayesian phylogenetic tree was constructed using the program MrBayes 3.1.2 [47] with models set for each partition based on jModeltest v0.1.1 [48]. The best-fitting model of sequence evolution based on the Akaike Information Criterion was the general time reversible model with Invariable sites (GTR+I). The Bayesian inference analysis was conducted by running two Markov Chain Monte Carlo searches each with 3 heated chains and 1 cold, starting from a random tree, proceeding for 5 million generations and sampling the chains every 1000 generations. Two independent runs were conducted to verify results. The first 20% of the

trees were discarded as burn-in. [44]. Posterior probabilities (PP) > 0.95 were considered as strong and PP < 0.80 were considered as weak support. For ML analysis, Tamura-Nei 3-parameter model [44] and Close-Neighbor-Interchange (CNI) algorithm was used in MEGA. To assess the robustness of the tree, 1000 bootstrap replicates were selected. Further, the maximum composite likelihood estimate of the pattern of nucleotide substitution for COI and tRNA/COII sequences of *A. gossypii* was performed using MEGA. Median joining networks were performed using NETWORK V.4.6.1.3 [49] to explore relationships among haplotypes of *A. gossypii* based on mitochondrial COI and COII/tRNA datasets.

RESULTS

Developmental and reproductive performance of *A. gossypii*

Clones of *A. gossypii* from various hosts showed significant differences in growth rate and reproductive rate on their respective host plants as presented in Table 2 and Table 3. Total nymphal duration (days) was in the order aubergine>okra, chili pepper>cotton and watermelon>cucumber. Total nymphal period of *A. gossypii* varied from 8.45 days on aubergine to 3.99 days on cucumber. The highest intrinsic rate of increase was determined on cucumber (r_m= 0.485), the lowest one was observed on aubergine (r_m= 0.253). The average longevity of adult females was reduced from 18.95 days on cucumber to 11.33 days on chili pepper. Maximum lifetime fecundity/female was observed on host plants of cucurbitaceae->malvaceae>Solanaceae. A similar trend of cucurbitaceae>malvaceae>solanaceae was observed for intrinsic rate of natural increase (r_m). Age-specific survival rate (l_x) and age specific fecundity (m_x) of *A. gossypii* on six different host plants in the laboratory is shown in Figure 1. Survival rates (l_x) of *A. gossypii* on the six different host plants were mainly affected by age and only slightly influenced by the host plant. Aphids confined to cucumber and watermelon hosts showed a distinct peak in nymph production (m_x) on 10th day, valuing 5.07 and 5.5 nymphs/female/day respectively. On cotton, the maximum number of nymphs/female/day was produced on 14th day followed by 16th, 18th and 12th for okra, aubergine and chili pepper respectively.

Host transfer experiments

Aphid clones on 6 different hosts could not survive when transferred to non–natal hosts between plant families (reciprocal transfers) and they all died in the first generation suggesting that *A. gossypii* on different host plants showed variation in their biological performances and could not establish on non-natal hosts. However, the success rate of colonization increased when aphids were repeatedly transferred to natal host plants.

Molecular analysis

Sequence analysis of mitochondrial COI sequences revealed 632 characters were conserved, 22 were variable of which only 4 characters was parsimony informative from the 658 bp region investigated. Correspondingly, 680 characters were conserved, 14 were variable of which only 8 characters was parsimony informative from the 702 bp region of tRNA/COII. The sequence comparison of partial mitochondrial COI (658 bp) gene exhibited a total variation of 0.19% among *A. gossypii* collected from six different crops representing three different plant families from various localities of India. Similarly, partial tRNA/COII (702 bp) exhibited a total variation of 0.17%.

The datasets for COI and tRNA/COII were combined based on the ILD test. The topologies obtained by BI and ML were similar, so only ML tree is shown (Figure 2). Bayesian posterior probabilities above 0.95 (left) and ML bootstrap over 80% (right) are indicated above the branches. The maximum composite likelihood estimate of the pattern of nucleotide substitution for combined sequences of COI and tRNA/COII of *A. gossypii* is given in Table 4. Substitution pattern and rates were estimated under the Tamura-Nei model [50]. The nucleotide frequencies for *A. gossypii* combined data set are 37.53% (A), 40.19% (T), 9.09% (C), and 13.20% (G). The base composition of the combined data set of COI and tRNA/COII gene fragments was biased toward Adenine (A) and Thymine (T). The overall transition (ti)/ transversion (tv) bias of *A. gossypii* was R = 2.37, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$. The maximum Log likelihood for maximum likelihood computation was -2282.49. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the datasets (complete deletion option in MEGA).

A total of 12 distinct haplotypes were identified based on COI sequences obtained from 36 individuals of *A. gossypii* collected on various host plants of Malvaceae, Cucurbitaceae and Solanaceae in India. There were 26 polymorphic sites of which 17 were transitional substitutions and 9 were transversional substitutions (Tables 5 & 6) (Figure 3A). Pairwise distance among COI haplotypes ranged from 0.15% (one nucleotide) to 1.22% (8 nucleotides). Similarly, a total of 15 haplotypes were identified based on COII/tRNA sequences with 21 polymorphic sites of which 10 were transitional substitutions and 11 were transversional substitutions (Tables 7 & 8) (Figure 3B). Pairwise distance among COII/tRNA haplotypes

ranged from 0.14% (one nucleotide) to 0.57% (4 nucleotides). The sequence divergence between haplotype H_1 and H_{11} was the largest based on COI and COII/tRNA datasets. Haplotype H_1 with maximum number of sequences (COI, COII/tRNA) indicated it is the most frequent one that exists in India and would likely be an ancester. There was no association between host plant families (Malvaceae, Cucurbitaceae and Solanaceae) neither with mtCOI haplotypes nor with mtCOII/tRNA haplotypes in India (Figure 3). Ancestral haplotype (H_1) was geographically widespread and observed to infest host plants irrespective of plant families.

Table 2. Developmental and reproductive performance of apterous female morph of *Aphis gossypii* on different host plants#

Biological attributes*	Malvaceae		Cucurbitaceae		Solanaceae	
	Cotton	Okra	Cucumber	Watermelon	Aubergine	Chili pepper
Nymphal period (d)						
1st Instar	1.07 ± 0.02a	1.50 ± 0.03b	1.00 ± 0.01c	1.11 ± 0.02a	2.09 ± 0.02d	1.57 ± 0.03e
2nd Instar	1.10 ± 0.03a	1.63 ± 0.02b	0.99 ± 0.01c	1.08 ± 0.02d	2.12 ± 0.03e	1.13 ± 0.03f
3rd Instar	1.15 ± 0.05a	1.93 ± 0.04b	1.00 ± 0.01c	1.10 ± 0.02c	2.08 ± 0.04d	1.82 ± 0.04e
4th Instar	1.07 ± 0.02a	1.13 ± 0.03a	0.99 ± 0.01b	1.05 ± 0.01a	2.17 ± 0.03c	1.54 ± 0.04d
Total Nymphal period (d)	4.31 ± 0.09a	6.20 ± 0.05b	3.99 ± 0.01c	4.33 ± 0.03a	8.45 ± 0.06d	6.07 ± 0.07b
Adult period (d)	18.07 ± 0.26a	15.14 ± 0.24b	18.95 ± 0.32c	16.82 ± 0.36d	12.48 ± 0.32e	11.33 ± 0.53f
Total life period (d)	22.38 ± 0.28a	20.21 ± 0.29b	22.94 ± 0.33a	21.14 ± 0.42b	20.94 ± 0.42b	17.40 ± 0.52c
Pre-reproduction period (d)	0.99 ± 0.01a	1.11 ± 0.04b	1.01 ± 0.01a	1.00 ± 0.01a	1.05 ± 0.02c	1.01 ± 0.01a
Reproduction period (d)	15.08 ± 0.26a	12.08 ± 0.21b	15.85 ± 0.33a	13.91 ± 0.32c	9.37 ± 0.33d	8.37 ± 0.51d
Post reproduction period (d)	0.99 ± 0.01a	1.01 ± 0.01a	1.05 ± 0.02b	0.98 ± 0.02c	1.03 ± 0.02b	1.02 ± 0.02b
Lifetime fecundity/female	41.33 ± 1.13a	24.00 ± 0.57b	60.93 ± 2.87c	60.67 ± 1.95c	26.53 ± 0.83b	20.20 ± 1.09b

Figures are mean ± SEM values of developmental and reproductive performance

*Sample size (n) is 15; Means within a row, followed by different letters are significantly different ($P < \mathbf{0.05}$; LSD)

Table 3. Biological performance of apterous female morph of *Aphis gossypii* on different host plants#

Biological attributes*	Abbr.	Malvaceae		Cucurbitaceae		Solanaceae	
		Cotton	Okra	Cucumber	Watermelon	Aubergine	Chili pepper
Net reproductive rate	R_o	38.04 ± 1.26a	23.59 ± 0.63b	60.11 ± 2.97c	63.89 ± 2.16c	24.79 ± 1.04b	29.30 ± 1.35b
Cohort generation time (d)	T_c	11.89 ± 0.14a	12.62 ± 0.13b	11.51 ± 0.25a	11.94 ± 0.16a	13.36 ± 0.17c	10.87 ± 0.22d
Innate capacity for increase	r_c	0.31 ± 0.01a	0.25 ± 0.01b	0.36 ± 0.01c	0.35 ± 0.01c	0.25 ± 0.01b	0.31 ± 0.01a
Generation time (d)	T	9.04 ± 0.11a	10.93 ± 0.11b	8.36 ± 0.15c	8.77 ± 0.08d	12.36 ± 0.13e	10.31 ± 0.15f
Finite rate of increase	λ	1.50 ± 0.01a	1.33 ± 0.01b	1.63 ± 0.02c	1.61 ± 0.01c	1.30 ± 0.01d	1.38 ± 0.01e
Doubling time (d)	DT	1.60 ± 0.01a	1.93 ± 0.01b	1.41 ± 0.03c	1.44 ± 0.01c	2.04 ± 0.01d	1.80 ± 0.01e
Gross reproductive rate	GRR	38.12 ± 1.31a	23.83 ± 0.66b	60.56 ± 2.99c	64.97 ± 2.40c	25.38 ± 1.26b	29.51 ± 1.97b
Intrinsic birth rate	b	2.47 ± 0.10a	1.95 ± 0.07b	4.47 ± 0.38c	4.10 ± 0.15c	2.55 ± 0.05a	2.88 ± 0.07a
Intrinsic death rate	d	2.08 ± 0.10a	1.67 ± 0.06a	3.97 ± 0.37b	3.56 ± 0.16b	2.30 ± 0.05a	2.55 ± 0.07a
Intrinsic rate of natural increase	r_m	0.41 ± 0.01a	0.29 ± 0.01b	0.49 ± 0.01c	0.47 ± 0.01c	0.25 ± 0.01d	0.33 ± 0.01e

Figures are mean ± SEM values of developmental and reproductive performance.

*Sample size (n) is 15; Means within a row, followed by different letters are significantly different ($P < \mathbf{0.05}$; LSD).

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Table 4. Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution for combined data set of COI and tRNA/COII sequences of *Aphis gossypii*

	A	T	C	G
A	-	*4.51*	*1.48*	**8.01**
T	*4.21*	-	**9.01**	*1.02*
C	*4.21*	**27.45**	-	*1.02*
G	**33.08**	*4.51*	*1.48*	-

Each entry shows the probability of substitution from one base (row) to another base (column) instantaneously. Only entries within a row should be compared. Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in italics. The overall average of pairwise genetic distance is 0.003.

Table 5. Variable position of 12 haplotypes of mtCOI gene sequence for *Aphis gossypii*

Haplotype	Nucleotide position beginning from 5' end of COI																								
	9	29	54	77	109	124	131	133	200	223	249	311	338	371	411	413	499	503	508	581	584	587	610	622	623	633
H_1	A	T	G	A	A	T	A	T	A	A	A	A	T	A	C	G	T	T	A	T	A	G	T	A	G	A
H_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	.
H_3	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
H_4	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	.	.	.	.	.	.	.
H_5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
H_6	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_7	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_8	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T
H_9	.	.	.	.	.	.	.	.	G	.	G	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.
H_{10}	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_{11}	G	.	T	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	T	A	T	A	.
H_{12}	.	.	.	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 6. COI haplotypes of *Aphis gossypii* revealed by construction of haplotype network. Sample numbers are the same as given in Table 1

Haplotype number	Number of sequences	Sample numbers
H_1	15	1–3, 5, 8–10, 13–16, 18, 24, 25, 27
H_2	10	19–22, 28–33
H_3	2	11, 12
H_4	1	7
H_5	1	6
H_6	1	4
H_7	1	17
H_8	1	23
H_9	1	36
H_{10}	1	35
H_{11}	1	26
H_{12}	1	34

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Table 7. Variable position of 15 haplotypes of mtCOII/tRNA gene sequence for *Aphis gossypii*

Haplotype	Nucleotide position beginning from 5' end of COII/tRNA																				
	25	35	36	66	95	114	160	190	219	232	343	373	397	460	495	521	572	574	621	648	694
H_1	T	A	A	A	T	T	A	A	A	C	G	T	T	C	G	A	G	A	T	T	T
H_2	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.
H_3	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
H_4	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
zH_5	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.
H_6	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_7	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
H_8	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
H_{10}	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_{11}	C	.	.	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_{12}	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
H_{13}	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
H_{14}	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	T	.	.	.	.
H_{15}	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	A	.	.

Table 8. COII/tRNA haplotypes of *Aphis gossypii* revealed by construction of haplotype network. Sample numbers are the same as given in Table 1

Haplotype number	Number of sequences	Sample numbers
H_1	13	1-6, 11, 12, 15, 16, 23, 24, 34
H_2	1	27
H_3	7	19, 21, 22 29-32
H_4	1	25
H_5	1	18
H_6	1	17
H_7	1	13
H_8	1	10
H_9	1	8
H_{10}	1	9
H_{11}	1	7
H_{12}	1	14
H_{13}	3	20, 28, 33,
H_{14}	2	35, 36
H_{15}	1	26

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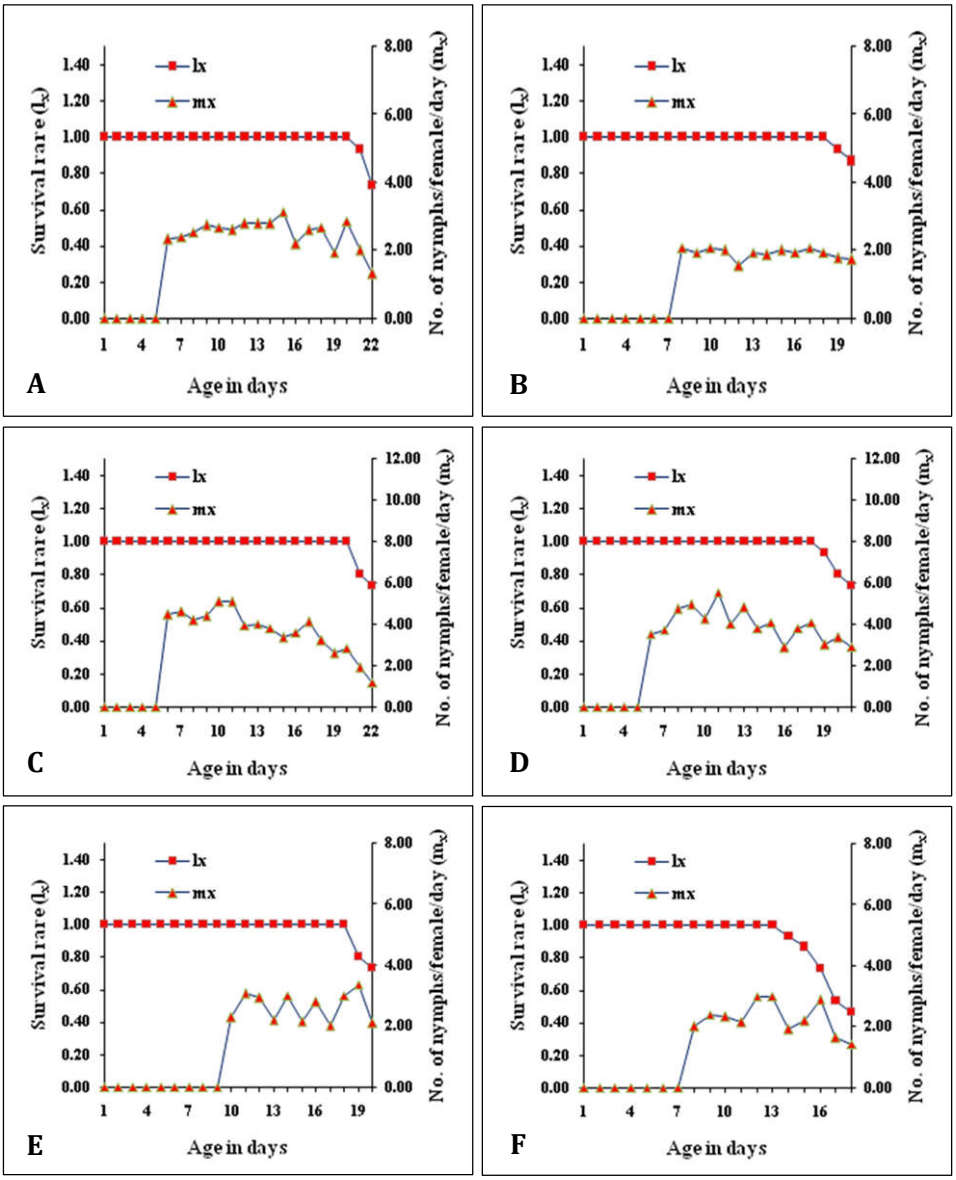

Figure 1 Age-specific survival rate (l_x) and age specific fecundity (m_x) of *Aphis gossypii* on six different host plants in the laboratory. Cotton (A), Okra (B), Cucumber (C), Watermelon (D), Aubergine (E) and Chili pepper (F).

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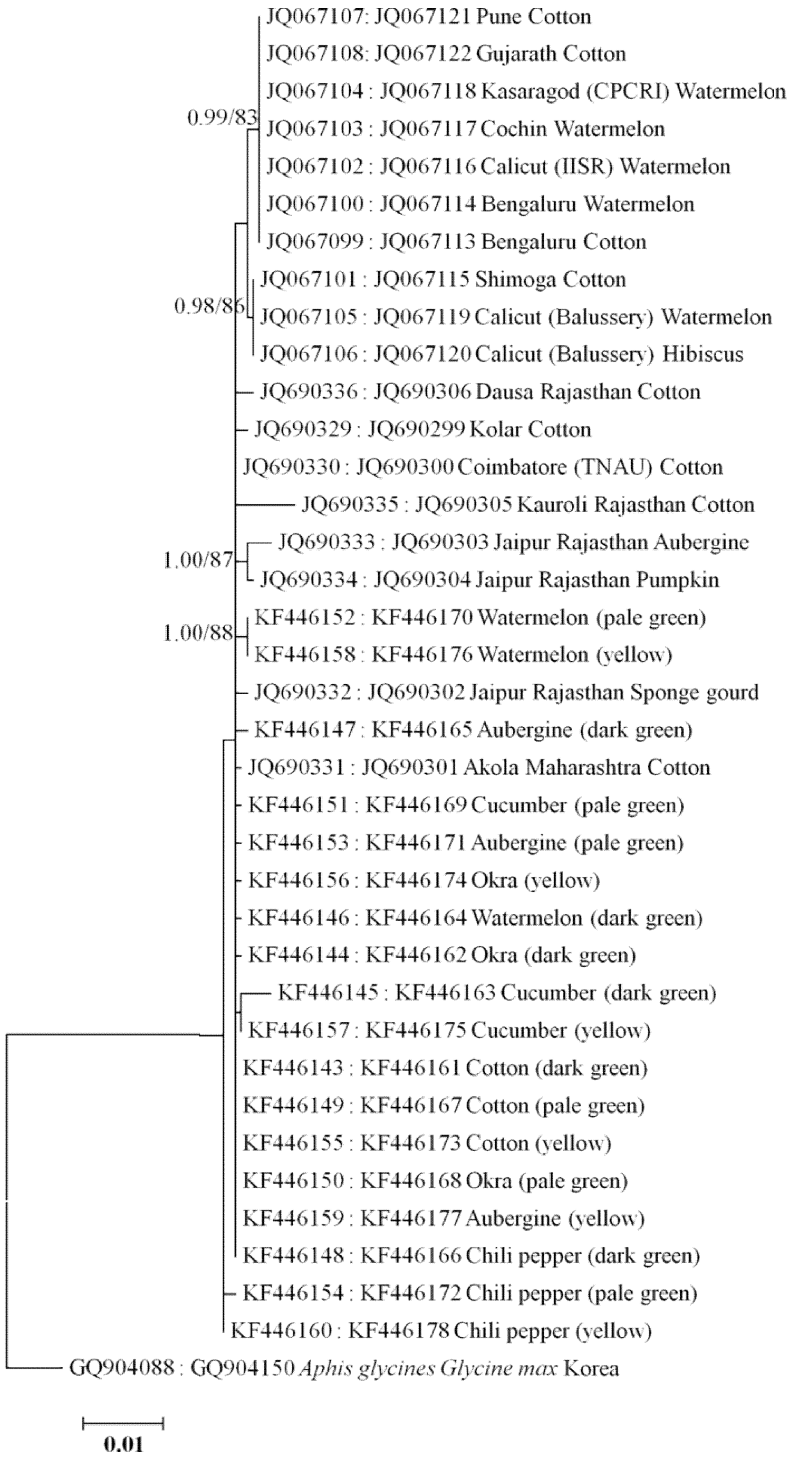

Figure 2 Maximum likelihood (ML) tree showing phylogenetic relationships among *Aphis gossypii* (collected from different host plants) based on combined sequences of COI (658 bp) and tRNA/COII (702 bp) with bootstrap support (1000 replicates). Posterior probabilities of BI analysis (left, >0.95) followed by ML bootstrap values (right, >80%) are shown for branches. *Aphis glycines* was used as outgroup.

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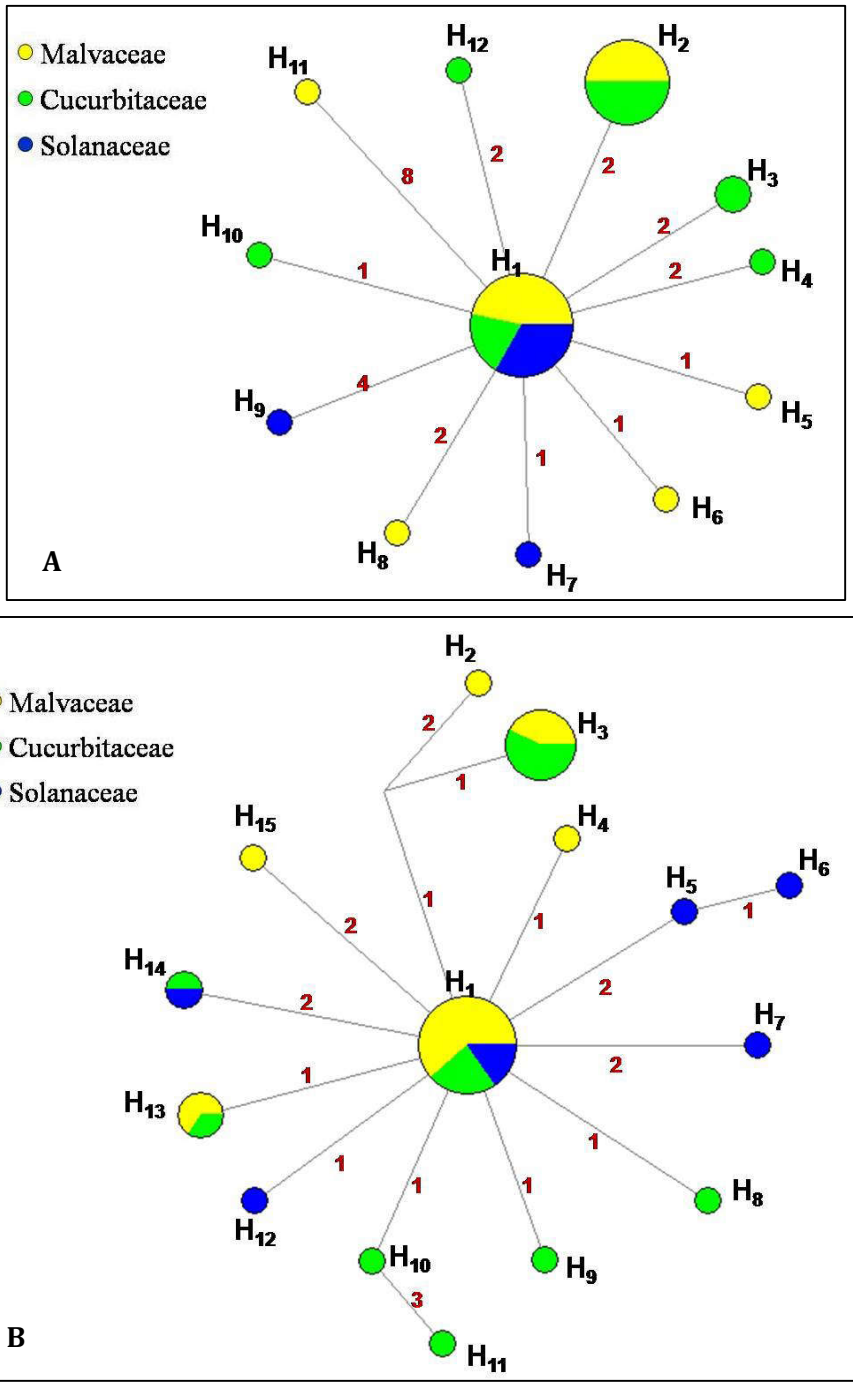

Figure 3 Median-joining networks based on *Aphis gossypii* mitochondrial COI (A) and COII/tRNA (B) datasets. Circles represent haplotypes obtained and the circle size represents its proportionality to the number of individuals sharing a haplotype. Numbers on branches represent the number of nucleotide substituted between haplotypes.

DISCUSSION

This study showed significant variation in biological performances of *A. gossypii* with respect to host plants. Nymphal duration varied significantly on cotton, okra, cucumber and aubergine. In contrast, adult longevity was aubergine>chili pepper, cotton>okra and cucumber>watermelon. Aphids showed a better performance on cucurbitaceous host plants [51, 52, 53] than on malvaceae and solanaceae. This was due to a short developmental period (4.0 days on cucumber and 4.3 days on watermelon) and high fecundity (3.21 and 3.60 nymphs/female/day) resulting in a high net reproduction rate (R_o=60.11 and 63.89 females/female). Reciprocal transfers were unsuccessful restricting their ability to colonize on non–natal

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host plants suggesting that conditioning was occurring in melon aphids as reported by researchers [17, 54, 55]. This provided the evidence for the presence of host-adapted genotypes/host races in laboratory clones of *A. gossypii*. However, the molecular analysis showed that the intra-specific variation was extremely low among *A. gossypii* clones infesting various host plants indicating a relatively recent divergence [26, 32, 56, 57]. The question of genetic versus conditioning factors in pea aphid host interactions is well addressed and concluded that genetic factors were of primary importance in determining the host specialization [17, 54]. Thus based on mtCOI and tRNA/COII sequence analysis there are no major host-associated genetic differences within Indian populations of *A. gossypii* as expected suggesting that it is a single cosmopolitan species [25, 26, 55]. In addition, network analysis revealed that there was no association between host plant families and mitochondrial haplotypes obtained in India. The ancestral haplotype H_1 was widely distributed on host plants irrespective of plant families and this could be attributed to regular genetic recombination and high behavioural plasticity [58]. An ongoing gene flow among *A. gossypii* populations on wide variety of host plants appears to be the major cause for the absence of host races in India. The differences in biology and behaviour observed on various hosts could be attributed to prior experience or seasonal factors influenced the ability of aphids to colonize particular plants [55], lack of dietary adaptation period that an insect need to undergo before establishing themselves on different host than the one from which they were isolated [51, 59], different phenology exhibited by host plants and a great variability in sap compounds such as nitrogen [60], secondary metabolites (e.g. alpha tomatine in tomato, solacidine in aubergines and minorcidine in cucurbits) [61] (Wink 2003), endosymbionts [62] and to unknown co-existing biotic factors that might act as selective factors for host acceptance by aphids [32]. Thus, the colonization and success of a polyphagous species such as melon aphid is a function of multiple factors.

The present study clearly showed that an ecological speciation driven by host plants is an ongoing phenomena and a relatively recent divergence in *A. gossypii* which requires constant monitoring. Since evolution of host specialization that results in genetically distinct sub-populations on different hosts is a complex process in aphids, it requires further investigation using microsatellite markers to draw a comprehensive knowledge on the host specificity of *A. gossypii*. These findings will aid in mapping the host-associated biotypes and effective utilization of biological control agents against this dreaded pest.

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COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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