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Research Article

Cytochrome oxidase subunit I (COI) revealed differentiation among populations of *Habrobracon hebetor* collected from various regions of Iran

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Abstract: *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is an ectoparasitoid wasp in the family Braconidae and is widely used in biological pest control. Little information is available on the genetic diversity of geographically isolated populations of *H. hebetor*. In the present study, we assess the genetic structure and diversity of geographically distinct populations of *H. hebetor* collected from different regions of Iran. To this end, 19 populations of *H. hebetor* (Dehloran, Hamadan, Minab, Rudan, Ahvaz, Sari, Semnan, Bandar Lengeh, Haji Abbad, Jiroft, Shiraz, Sarpol-e Zahab, Gorgan, Isfahan, Urmia, Kahurestan, Taziyan, Isin, and Sarkhun) were collected from natural niches. For each population, we sequenced a ~660 base pair fragment of Cytochrome Oxidase subunit I (COI) successfully. Analysis of molecular variance revealed sharp differentiation among *H. hebetor* populations. Populations from Ahvaz, Dehloran, Jiroft and Minab were the most genetically diverged. A Mantel test showed significant positive correlation between genetic and geographic distances ($r = 0.47$, $P < 0.001$). The phylogenetic analysis clustered the populations into two major groups (A and B) (100); the major part was assigned to group A. Group B mainly included the populations from southern Iran. Based on these results, we conclude that *H. hebetor* in Iran is comprised of many diverse populations. These may be successfully applied in innundative release programs.

Keywords: Genetic structure, various regions, Haplotype diversity, Bayesian inference

Introduction

Parasitoids can play a crucial role in integrated pest management (IPM) programs to keep pest populations below the economic levels (Belda and Riudavets, 2013). *Habrobracon hebetor* Say (Hymenoptera: Braconidae) is an important biological control agent that has been widely used against lepidopteran insect pests in inundative release programs (Antolin *et al.*, 2003; Chen *et al.*, 2011; Alam *et al.,* 2016; Razmjou *et al.,* 2018; Bagheri *et al.,* 2019; Badran *et al.,* 2021). There is a need for evolutionary and phylogenetic studies that will assist biological control practitioners to more effectively exploit intraspecific genetic variation and micro-evolution to benefit pest management (Phillips *et al.,* 2008.). Therefore, to

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maximize the effectiveness of the control programs, it needs to study various biological and genetic aspects of a biological control agent like *H. hebetor* to focus on a more diverse and efficient population. It has been revealed that populations of *H. hebetor* with different geographical and ecological niches differed in female longevity, sex ratio, linear sizes of imago and cocoon, the color of imago, motor activity, and the nature of oviposition (Statkevych and Drozda, 2020). In addition, they had different food preferences and conditions of breeding (Kil *et al.,* 2018). Koohpayma *et al.* (2019) characterized life-history traits of *H. hebetor* populations collected from climatically and geographically isolated regions of Iran and found significant differences in the female longevity, paralysis, and parasitism rate, sex ratio, reproductive rate, and host allocation among these populations (Koohpayma *et al.,* 2019). Kil *et al.* (2018) studied two geographic populations of the *H. hebetor* collected from Krasnodar, Russia, and Chimkent, Kazakhstan, using RAPD markers and showed they had relatively high genetic distance and very low gene drift. The populations bunched in two different clusters according to their geographic isolation. Chomphukhiao *et al.* (2018) studied Thai and Japanese populations of *H. hebetor* using COI and 16S markers and showed that Thai populations were genetically different from Japanese.

Intra and inter-population genetic variations are two critical issues that need to be assessed in natural enemies before inundative release programs (Grenier, 1988; Van Lenteren, 2000). Because this information will help us to have a successful inundative release either by assisting the collection and release of wide variation to enhance the probability of released agents will become locally adapted or by helping to identify the subset of genetic variation most suitable for release (Wajnberg, 2004; Kil *et al.,* 2018; Mangan *et al.,* 2019; Cuthbert *et al.,* 2020). Despite valuable information on various biological aspects of *H. hebetor*, little is known about the genetic structure of its populations. Previous studies have been restricted to local studies or a low number of locations (Chomphukhiao *et al.,* 2018; Kil *et al.,* 2018; Statkevych and Drozda, 2020). Garba *et al.* (2019) studied the genetic structure of *H. hebetor* and found only moderate genetic differentiation among populations of *H. hebetor* collected from Niger and Iran.

Molecular genetic methods are widely used to evaluate insect population structure (Lozier *et al.,* 2009; Uddin and Tsuchida, 2012; Costa *et al.*, 2021; Wachi *et al.*, 2021). They can also be utilized for determining intra- and interspecific diversity (Kazachkova *et al.,* 2008; Samara *et al.,* 2008). Mitochondrial-based genetic markers have been used abundantly by different researchers to study taxonomic problems and to study biological traits such as host specificity and dispersal ability in natural enemies through assessing population structure (Muirhead *et al.,* 2012; Taylor *et al.,* 2011; Rauth *et al.,* 2011; Barbosa *et al.,* 2014). They have also been used in the study of genetic diversity (Kavar *et al.,* 2006; Lewter *et al.,* 2006; Schroer *et al.,* 2008; Mugerwa *et al.,* 2012; Palomera *et al.,* 2012), phylogeny (Smith and Gaffney, 2005; Ito *et al.,* 2011), phylogeography (Faccoli *et al.,* 2005; Meng *et al.,* 2008; Ballman *et al.,* 2011), cryptic species (Schutze *et al.,* 2006; Williams *et al.,* 2006; Cifuentes *et al.,* 2011; Zhou *et al.,* 2012) and host-related genetic differentiation (Dorchin *et al.,* 2009; Mezghani-Khemakhem *et al.,* 2012).

Our previous studies have shown that the geographically isolated populations of *H. hebetor* were different in terms of life-history traits (Koohpayma *et al.,* 2019). Here, we aimed to study the genetic diversity of geographically isolated populations of *H. hebetor* collected from different regions of Iran. This information could improve the effectiveness of inundative release programs by focusing on more genetically diverse populations of *H. hebetor*.

Materials and Methods

Field sampling

Habrobracon hebetor populations were sampled from across the native range of the species in Iran in two consecutive years (2017 and 2018). Samples were obtained from 19 geographically distinct

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locations (Dehloran, Gorgan, Urmia, Hamadan, Rudan, Minab, Hajiabad, Bandar Lengeh, Jiroft, Sarpol-e Zahab, Shiraz, Ahvaz, Sari, Semnan, Isfahan, Taziyan, Sarkhun, Kahurestan and Isin) (Fig. 1; see Table 1 for precise localities), distributed over eight climatic zones (Table 2) of Iran. At each sampling site, thirty transparent plastic cages (7.5 × 5.5 × 9.5 cm) were placed in agricultural crop fields (tomato, garden pea, corn, chickpea, okra, cucumber, cabbage, sweet pepper, palm, mango, citrus, and apple) as well as in rangelands. Ten larvae of the fourth and fifth instar, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), were enclosed between two layers of netting and put on the open side of the plastic cage, and then placed in the field for 24 h to trap parasitoids. The parasitized larvae were incubated in a growth chamber under 25 ± 1 °C, 65 ± 5 RH, and 16:8 h (L:D) until the emergence of the *H. hebetor* adults. Freshly emerged females were stored at -80 ºC to avoid DNA degradation up to the moment of DNA extraction.

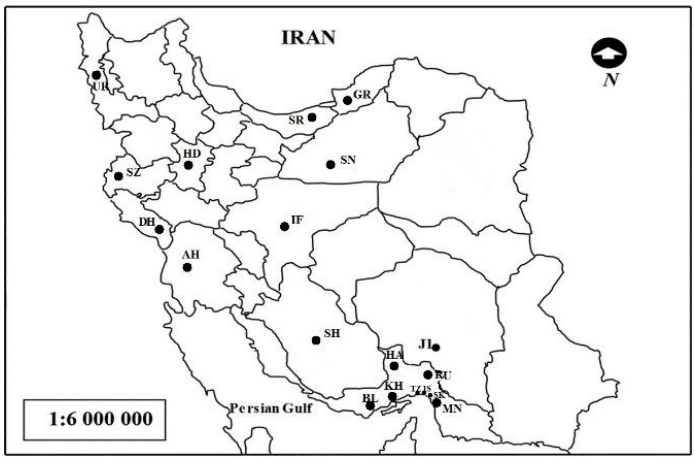

Figure 1 Map of Iran. Identification codes on the map refer to the sampling points of the *Habrobracon hebetor*. GR, Gorgan; SR, Sari; HD, Hamadan; UR, Urmia; SZ, Sar pol-e Zahab; DH, Dehloran; AH, Ahvaz; SN, Semnan; JI, Jiroft; SH, Shiraz; HA, Haji Abad; KH, Kahurestan; BL, Bandar Lengeh; MN, Minab; RU, Rudan; TZ, Taziyan; IS, Isin; SK, Sarkhun; IF, Isfahan

Table 1 Localities of sample collection sites for *Habrobracon hebetor* populations, codes and coordinates of collection sites, and number of individuals used in the analysis (COI).

Population identification	Collection site	Longitude, latitude	Elevation	Individuals used in analysis (COI)
BL	Bandar Lengeh	54°51'20.73"E, 26°31'57.98"N	*6*	*2*
TZ	Taziyan	56°10'45.07"E, 27°18'50.03"N	85	1
DH	Deloran	47°15'18.44"E, 32°41'30.01"N	207	8
SZ	Sarpol-e Zahab	45°51'0.78"E, 34°27'18.91"N	2977	8
HD	Hamadan	48°27'34.63"E, 34°46'12.97"N	2038	11
UR	Urmia	45° 7'10.73"E, 37°32'45.10"N	4325	10
GR	*Gorgan*	54°28'46.49"E, 36°51'25.09"N	*80*	*10*
MN	Minab	57°2'23.15"E, 27°8'37.09"N	34	10
HA	Haji Abad	55°45'10.95"E, 28°16'57.30"N	877	9
JI	Jiroft	57°48'45.48"E, 28°38'15.54"N	672	8
RU	Rudan	57°4'54.41"E, 27°28'53.77"N	313	5
SH	Shiraz	52°34'59.68"E, 29°33'52.92"N	1497	9
IF	Isfahan	51°34'13.73"E, 32°37'6.13"N	1596	19
AH	Ahvaz	48°25'57.20"E, 31°28'31.43"N	23	10
SR	Sari	53°12'2.42"E, 36°38'6.34"N	12	14
SK	Sarkhun	50°33'0.12"E, 31°44'32.35"N	72	6
SN	Semnan	54°23'42.28"E, 36°7'56.05"N	1101	11
KH	Kahurestan	55°34'19.34"E, 27°12'45.02"N	42	2
IS	Isin	56°12'51"E, 27°18'57"N	68	3

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Table 2 Description of the trapping locations of the populations including climate and vegetation.

Populations	Zone	Climate	Description of the location	Vegetation
Dehloran	1	Warm and temperate with a lot of rain	In the Ilam province	Agriculture farms (tomato, okra, chickpea and corn)
Sar pol-e Zahab	1	Mild and generally warm and temperate with a lot of rain	In the Kermanshah province	Agriculture farms (tomato, peas and corn)
Semnan	1	Mild and generally warm and temperate with a lot of rain	In the Semnan province	Agriculture farm (Corn)
Hamadan	2	Cold semi-arid climate with snowy winters	In the Hamadan province	Agriculture farms (tomato, corn, cucumber) and orchard (Apple)
Urmia	2	Cold semi-arid with cold winters	In the West Azerbaijan Province	Agriculture farms (chickpea) Orchard (Apple)
Sari	3	Wet forests along the Caspian sea coast	Mazandaran province, Sari city, Dashte Naz in coastal Caspian sea	Agriculture farms (tomato) and orchard (Citrus)
Gorgan	3	Wet forests	In the Gorgan province	Agriculture farms Rapeseed and garden Pea
Ahvaz	4	Very hot and occasionally humid with cold and dry winters	In the Khuzestan province, Ahvaz city	Agriculture farms (cabbage and cucumber)
Isfahan	5	Dry and hot climate with cold winters	In the Esfahan north Bra an region, along the Zayandeh_rood river	Agriculture farms (cabbage and chickpeas)
Shiraz	6	Warm and dry with cool winters	In the Fars province	Agriculture farms (corn) and orchard (palm, pomegranate)
Haji Abad	6	Warm and dry with cool winters	In the Hormozgan province, Haji Abad city, along the waterfall	Orchard (palm)
Jiroft	6	Warm and dry with cool winters	In the Kerman province	Agriculture farm (tomato)
Sarkhun	7	Very hot and humid with mild and occasionally cool summer	In the Hormozgan province, Bandar Abbas city, close of Persian Golf	Rangeland and agriculture farm (Sweet peppers)
Taziyan	7	Very hot and semi-drid with mild and occasionally cool summer	In the Hormozgan province, Bandar Abbas city, close of Persian Golf	Rangeland
Minab	7	Very hot and humid with mild and occasionally cool summer	In the Hormozgan province	Orchard (citrus, palm and mango)
Bandar Lengeh	7	Very hot and humid with mild and occasionally cool summer	Gesheh village, along to Persian Golf	Agriculture farms (tomato) and orchard (palm)
Kahurestan	7	Very hot and humid with mild and occasionally cool summer	In the Hormozgan province, Bandar Khamir city and close of Mangro forest	Agriculture farms (tomato) and rangeland
Isin	7	Very hot and humid	In the Hormozgan province, Bandar Abbas city and close of Persian Golf	Rangeland and agriculture farms (tomato)
Rudan	8	Very hot and dry	In the Hormozgan province, Dashte Naz	Orchard (citrus)

DNA extraction and sequencing

DNA was extracted from individual adult females using the cetyl trimethyl-ammonium-bromide (CTAB) method following the protocol outlined by Reineke *et al.* (1998). Each DNA sample was dissolved in 50 µl D. D. W and stored at -20 ºC till use. The quality and quantity of the extracted DNA were checked by running each sample on an agarose gel (1%) and using Nanodrop (Thermo NanoDrop 1000), respectively.

A 685 base pair fragment of cytochrome C oxidase subunit I (COI) was amplified in all individuals by polymerase chain reaction (PCR) using the primer pair LCO 1490 (5'-GGTCAA CAAATCATAAAGATATTGG-3')/HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.,* 1994; Heimpel *et al.,* 1997). Each PCR mixture contained 12 µL of Amplicon master mix, 10 µL of D.D.W, 1 µL of each oligonucleotide primer (100 µM), and ~ 100 ng genomic DNA template. The PCR was performed in an Eppendorf thermocycler programmed at 94 °C for two minutes, five cycles of 94 °C for 30 seconds, 45 °C for 50 seconds, 72 °C for 40 seconds followed by 35 cycles of 94 °C for 30 seconds, 51 °C for 30 seconds, 72 °C for 40 seconds, and a final extension at 72 °C for two minutes (Penton *et al.,* 2004). PCR products were subjected to electrophoresis on 1% agarose gel and stained with SYBR Green (SYBR safe CinnaGen, Tehran, Iran). All PCR products were sequenced by Macrogen Sequencing Service (Seoul, South Korea). The sequence data were deposited in the GenBank database under accession numbers MK604070–MK604141, MK350267–MK350279, MK350281–

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MK350290, MK867764–MK867771, and MK376137–MK376189.

Data analysis

We tested for a correlation between geographic and genetic distances by performing a partial Mantel test within isolation by distance web service (IBDWS) version 3.23 (Jensen *et al.,* 2005). Genetic differences of populations (Nei and Li's index) were used for principal coordinate analysis using the ape package (Paradis *et al.,* 2004) in R version 3.1.0 (cran.r-project.org). To visualize the similarities of populations, the first three coordinates were plotted pairwise.

Multiple alignments of the sequences were performed with ClustalW in Mega 4.1 (Kumar *et al.,* 2008). The same program was also used to assess nucleotide composition and variable sites. Genetic diversity and standard deviations (SD) were estimated for haplotype diversity (Hd) and nucleotide diversity (π) in DnaSP version 4.10.1 (Librado and Rozas, 2009).

The number of individuals (N), haplotype (h), haplotype diversity (Hd, mean ± SD), nucleotide diversity (π, mean ± SD), and the number of pairwise differences (MNPD, mean ± SD) were computed using Arlequin version 3.5 (Excoffier and Lischer, 2010). Evolutionary relationships among haplotypes were represented by constructing a haplotype network of the COI sequences based on the statistical parsimony method (Templeton *et al.,* 1992) using TCS version 1.21 (Clement *et al.,* 2000).

Analysis of molecular variance (AMOVA) was carried out using Arlequin version 3.5 (Excoffier and Lischer, 2010). The significance level of F_{ST} statistics was assessed using a non-parametric permutation procedure with 1023 randomization in Arlequin. Nei's standard genetic distance was computed using the same program. Tajima's *D* and Fu and Li's *D* statistics were assessed for deviation from neutrality within populations using DnaSP. Statistical calculations and graphics for F_{ST} (Weir and Cockerham, 1984) were conducted using R.

The model of base substitution was chosen using MrModeltest2 (Nylander, 2004). Based on the Akaike criterion, a general time-reversible model, including among-site rate heterogeneity and estimates of invariant sites (GTR + G + I), was used in phylogenetic analyses. The phylogenetic tree was inferred using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). After discarding burn-in (25% of the samples) samples and evaluating convergence, the remaining samples were retained for further analysis. The Markov chain Monte Carlo (MCMC) method within a Bayesian framework, run for 10 million generations, was used to determine the equilibrium distribution and to estimate the Bayesian posterior probabilities (BPP) of groups (Larget and Simon, 1999) using the 50% majority rule. The BPP values higher than 0.50 are given on appropriate groups. Output phylogenies were visualized and re-drawn using Dendroscope V.3.2.8 and CorelDRAW V. X7, respectively.

Results

A ~660 base pair fragment of the COI gene was successfully sequenced for 156 *H. hebetor* individuals. The final alignment of the amplified mtDNA COI gene region defined a 544 base pair fragment present in all individuals studied. Nineteen different haplotypes were identified. The haplotypes contained 44 polymorphic nucleotide sites from a total of 544 (nucleotide diversity 0.01604). The final alignment of the COI sequences comprised 44 polymorphic nucleotides, of which 18 were parsimony informative sites. The proportion of the various haplotypes resulted in a high Hd of 0.5620, while nucleotide diversity (π) (0.01604) was generally low. Analysis of molecular variance showed significant differences among *H. hebetor* populations ($P < 0.01$) with high between-population variation (92.35). F_{ST} values calculated based on the COI alignment showed that Ahvaz, Jiroft, Minab populations were differed significantly from the other populations and exhibited the most between population variation (see Figs. 2-3). Less

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genetic variation was found within populations (7.65%) (Table 3). Kahurestan, Isin and Shiraz populations contained the most within-population variations (Fig. 3). Result of Tajima's D and Fu's test for the total samples was not statistically significant (Tajima's D = 0.20251, P > 0.1; Fu and Li's D = 0.60562, P > 0.1; Fu and Li's F = 0.52189, P > 0.1). The partial COI sequences of 156 *H. hebetor* individuals and three sequences from GenBank (KY484509, KY271883, MH766533) were used for the further phylogenetic analyses. A sequence from GenBank (AB456706) was also used as outgroup. All sequences from Dehloran, Hamadan, Minab, Sari, Semnan, Bandar Lengeh, Haji Abbad, Jiroft, Sarpol-e-Zahab, Gorgan, Isfahan, Urmia, Isin, Rudan and Sarkhun populations are in group A. Besides the mentioned populations, three sequences from the Shiraz population (SH3, SH4, SH9) were also placed in this group. The genetic relationship of these populations was supported by relatively low Bayesian posterior probabilities (0.61). Group B consisted of 25 sequences, including 10 sequences from Ahvaz (AH1–AH10) and five from the Shiraz population (SH1, SH2, SH5, SH7, SH8) diverged from a sequence from Shiraz (SH6). This group was also supported with low Bayesian posterior probabilities (0.58) (Fig. 4).

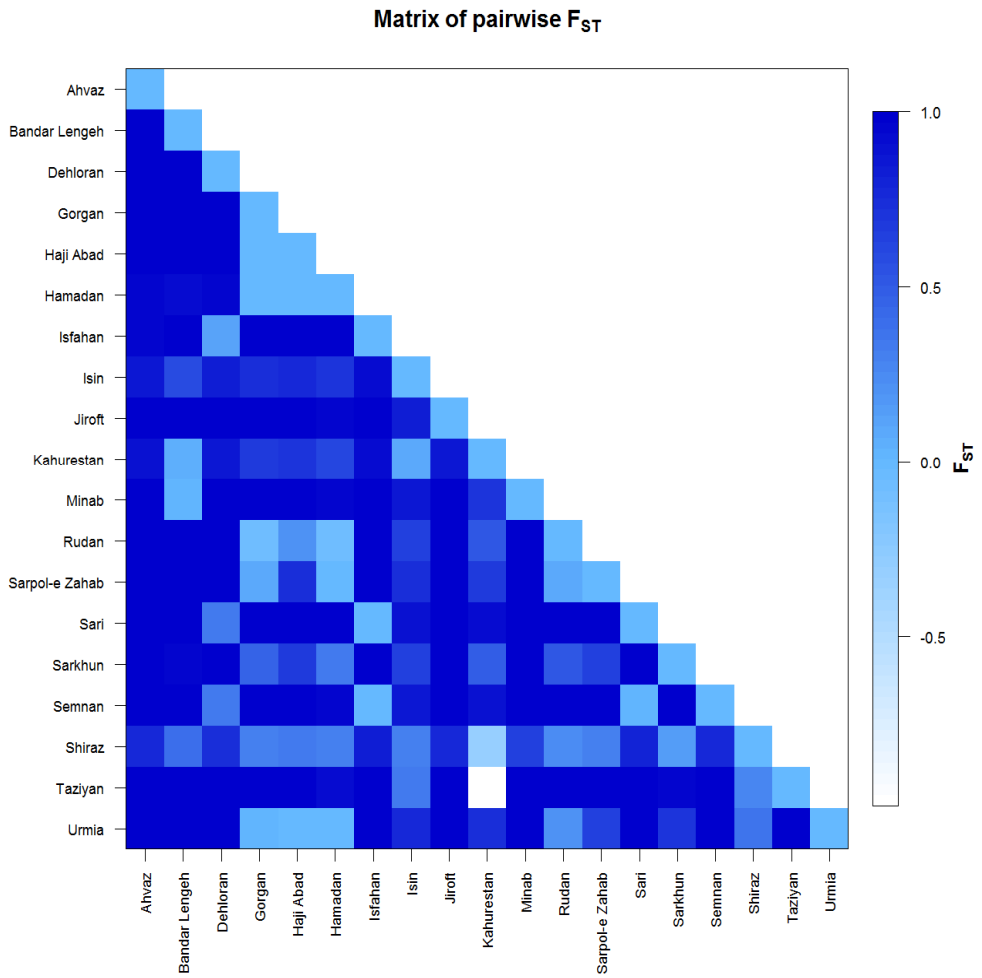

Figure 2 Population pairwise F_{ST} of comparison of the 19 geographical populations based on a COI gene.

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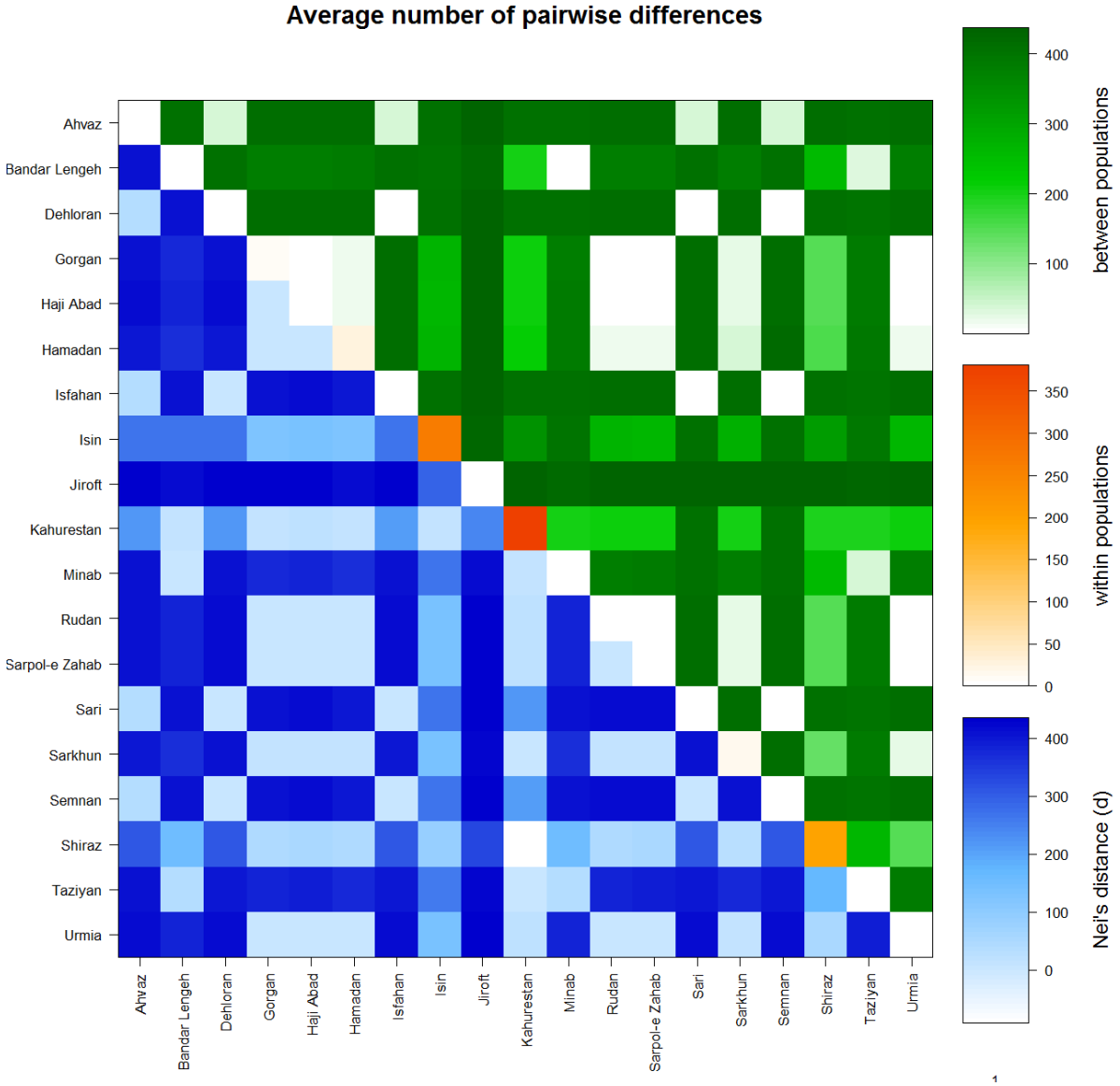

Figure 3 Average number of pairwise differences and Nei's distance within and between the 19 *Habrobracon hebetor* populations obtained from COI gene sequences.

Table 3 AMOVA results comparing genetic variation in *Habrobracon hebetor* collected from 19 localities.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation index
Between populations	14	127.757	2.25695 Va	92.35	F_{ST} = 0.65081
Within populations	38	46.017	1.21096 Vb	7.65	P < 0.01
Total	52	173.774	3.46791		

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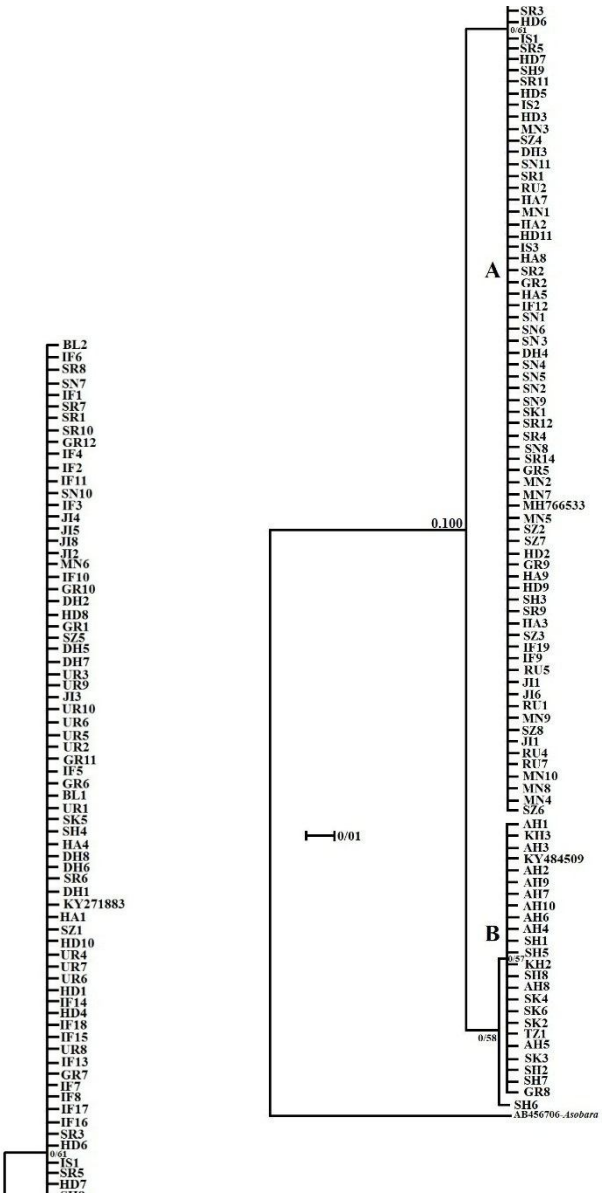

Figure 4 The 50% majority rule consensus tree inferred from Bayesian analysis of 156 cytochrome oxidase I under the GTR + G + I model. The phylogenetic tree reveals that sixteen populations clustered in group A with low Bayesian posterior probabilities (0.61), including Rudan, Minab, Isfahan, Dehloran, Hamadan, Sari, Sar pole Zahab, Gorgan, Bandar Lengeh, Jiroft, Urmia, Haji Abad, Isin Sarkhun and Semnan, along with two sequences from Shiraz. group B includes 24 sequences from GenBank and one sequence from India with low Bayesian posterior probabilities (0.58). Group B has 10, 6, 4, 2, 1, and 1 sequences from Ahvaz, Shiraz, Sarkhun, Kahurestan, Taziyan and Gorgan, respectively, and is monophyletic (0.94). Bayesian posterior probabilities values are given for the appropriate group. The newly sequenced individuals are indicated in bold**.**

Nineteen haplotypes (including HT1–HT19) with 44 polymorphic sites were observed in 19 *H. hebetor* populations studied. The haplotypes HT1, HT3, HT5, HT6, HT7, HT8, HT9, HT10, and HT13 were found in more than one population. HT1 was the dominant haplotype comprising 65.38% of all sequences and was shared by 16 populations. HT6 was shared by the

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Dehloran, Jiroft, Sar pol-e Zahab and Gorgan populations. The HT3, HT7, and HT13 haplotypes were each shared by three populations. The samples collected from Shiraz showed the most haplotype diversity (six) and the highest nucleotide diversity. In addition, four haplotypes were only found in the samples collected from Ahvaz. This population also showed high nucleotide diversity (Table 4).

The result of the Mantel test showed a significant correlation between genetic distances and geographic distances ($r = 0.47$, $P < 0.001$).

Table 4 Distribution and frequency of 19 mitochondrial haplotypes (HT1–HT19) with 44 polymorphic sites in different populations of *Habrobracon hebetor*.

HT	AH	DH	TZ	SR	SK	SN	SZ	UR	JI	IS	IF	GR	HA	HD	MN	RU	SH	KH	BL
HT1		4		13	1	8	6	9	2	2	18	6	9	11	6	3	2		2
HT2															1				
HT3						1			1			1							
HT4															3				
HT5				1		1													
HT6		4					2		5			1							
HT7					1											1	1		
HT8	3											1							
HT9	3		1		4												2	1	
HT10								1			1								
HT11	2																		
HT12																1			
HT13	1																2	1	
HT14				1															
HT15																	1		
HT16										1									
HT17																	1		
HT18						1													
HT19	1																		

Note: See Table 1 for the details of the sampled localities.

Discussion

The results of the present study demonstrated high inter-population differentiation among 19 populations of *H. hebetor* from different regions of Iran. These populations of *H. hebetor* are grouped into two separate clusters. These findings are consistent with other researchers who showed climatically distinct populations of insects were genetically different from each other (Baker *et al.,* 2003; Spielman *et al.,* 2004). Our results suggest that climate, geographic isolation, and habitat can affect the genetic structure of insect populations in the long term. Liang *et al.* (2008) stated that the limited gene exchange among *Cordyceps sinensis* populations caused by the geographic isolation led to a high genetic differentiation among populations on a regional scale (in each group, the intra and inter-population variation was low). Their results showed a high polymorphism rate among populations collected from different geographical regions. In addition, the grouping pattern seems to match with the geographic distribution along a latitudinal gradient. Hedrick *et al.* (1976) and Nevo *et al.* (1988) indicated that spatiotemporal isolation could preserve genetic diversity. Moreover, genetic variation among populations and patterns of gene flow may reflect underlying discontinuities in available habitats. These discontinuities can be in either space or time (1) and provide a null model to test additional hypotheses. The relationships among

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genotypes (phylogeny of alleles or haplotypes) from one or more population(s) can be examined relative to their geographical location (Roderick, 1996).

A total of 19 haplotypes were found among the 19 populations, and populations differed in their haplotype frequency. The Shiraz, Ahvaz, and Gorgan populations were the most diverse, with six, five, and four haplotypes. The other populations had one, two, or three haplotypes. In addition, the within-population variation was considerably low in the latter mentioned populations. The low within-population genetic variation can imply strong selection imposed by intensive chemical control in these populations, causing the elimination of susceptible haplotypes. Theoretically, there are four different ways in which toxicants can affect genetic variation: (i) by increasing mutation rates, (ii) by directional selection on tolerant genotypes, (iii) by causing bottleneck events, and (iv) by altering migration (van Straalen and Timmermans, 2002). Field assessment regarding the populations with low genetic diversity corroborated the presence of strong selection pressure in their sampling sites. The negative effect of selection pressures such as an intensive chemical application on the number of haplotypes in natural enemies has been documented by other researchers (Bagheri *et al.,* 2018; Brown *et al.,* 2009; Chang *et al.,* 2016). The loss of genetic diversity would increase the susceptibility of populations to become endangered (Frankham, 2003) and reduce the ability of populations to respond evolutionarily to environmental change (Frankel and Soulé, 1981). The loss of genetic diversity in populations experiencing anthropogenic stress can be designated as "genetic erosion" and may be a factor of concern in the risk assessment of toxic chemicals (van Straalen and Timmermans, 2002).

Our results revealed that some Iranian populations grouped with samples from India, Australia and Russia. Apart from that, they may have originated from a very prevalent ancestor; this may also imply the same view regarding the control of insect pests, strongly emphasizing insecticide application (Braccia and Voshell, 2005; Hoffmann and Sgro, 2011).

The phylogenetic tree inferred from COI sequences grouped all populations into two major distinct groups, in which all Ahvaz, Kahurestan, Taziyan individuals and more individuals of Shiraz and Sarkhun grouped in group B. In contrast, the other Iranian populations grouped in group A. Populations clustered in group B shared a haplotype with a sequence from an Indian population (KY484509) retrieved from GenBank. None of the Kahurestan, Ahvaz and Taziyan populations had the HT1 haplotype, which may reflect the incompleteness of sampling (Bagheri *et al.,* 2018), higher resistance of existing haplotypes to insecticides (Piiroinen *et al.,* 2013), or removal of haplotypes by chemical controls. All the other remaining populations in group A shared the HT1 haplotype, showing a regular gene flow between these populations. Gene flow in conjunction with frequency-dependent selection would slow up the potential loss of alleles because of genetic drift. All individuals from Bandar Lengeh, Gorgan, Jiroft, Urmia, Haji Abad, Rudan, Minab, Isfahan, Dehloran, Hamadan, Sari, Sar pole Zahab, Semnan, and four individuals from Sarkhun (SK1, SK5) and Shiraz (SH3, SH4) clustered in group A, indicating the occurrence of a common founding ancestor. Analysis of COI sequence retrieved from GenBank allied these sequences besides our sequences having HT1 haplotype and confirmed that our sampling has been comprehensive enough because our sampling shared common haplotype with haplotypes that have been reported from other countries.

Interestingly, we found that populations with high genetic diversity (Kahurestan and Bandar Lengeh) were already represented by their higher parasitism efficiency compared to other populations (Koohpayma et al., 2019), which may show a positive correlation between increased genetic diversity and parasitism performance in *H. hebetor* populations. The opposite was also true, and low diverse populations had lower parasitism performance. In addition, our results revealed high genetic diversity in populations

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collected from tropical and subtropical regions (like Ahvaz). Many *H. hebetor* populations in these regions were collected from date orchards and rangelands less experienced chemical spraying. It has been revealed that climatic differences or geographical barriers might weaken the capacity of some *Cotesia sesamiae* populations. To colonize areas recently invaded by a host is suitable for parasitoid larval development, although parasitic wasps have been shown to disperse quite efficiently, sometimes beyond the capacity of their associated host (Branca *et al*., 2019).

Some geographically close populations (Ahvaz and Dehloran populations) were genetically different and grouped in different groups. This difference was confirmed by the results of the Mantle test, in which populations with more geographic distance were genetically distinct from each other. The inability of populations to fly long distances (Timmermans *et al.,* 2005; Franck and Timm, 2010) may explain this result because it can promote divergence of populations due to genetic drift or intensive pesticides application (Loaiza *et al.,* 2010). de León and Jones (2005) studied geographically distinct populations of the parasitoid *Gonatocerus ashmeadi* (Girault) (Hymenoptera: Mymaridae) using ISSR–PCR markers and found it has high genetic differentiation and genetic diversity.

Conclusion

In conclusion, our study revealed considerable genetic variation among *H. hebetor* populations, which may stem from a geographic-based divergence among these populations. This divergence may also be intensified by climatic differences between populations and impact life-history traits of *H. hebetor*. Strong genetic and phenotypic population differentiation may explain a high discrepancy between results of various researchers that have studied life-history traits of *H. hebetor* under the same conditions. Also, these results can help producers to focus on populations with high genetic diversity that may have superior performance in terms of parasitism performance.

Conflict of interest

The authors declare that they have no conflict of interest.

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زیرواحد سیتوکروم اکسیداز یک (COI) تفاوت زیادی را در بین جمعیت‌های مختلف آب و هوایی *Habrobracon hebetor* (Haymenoptera: Braconidae) نشان داد

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چکیده: زنبور *Habrobracon hebetor* Say (Hymenoptera: Braconidae) یک اکتوپارازیتوئید از خانواده براکونیده بوده که به‌صورت گسترده در کنترل بیولوژیکی آفات مورد استفاده قرار می‌گیرد، گرچه اطلاعات کمی از تنوع ژنتیکی جمعیت‌های مختلف آب و هوایی *H. hebetor* وجود دارد. در این مطالعه ساختار ژنتیکی و تنوع ژنتیکی جمعیت‌های مختلف *H. hebetor* از نظر تفاوت‌های مختلف جغرافیایی و آب و هوایی ارزیابی شد. در پایان ۱۹ جمعیت *H. hebetor* (دهلران، همدان، میناب، رودان، اهواز، ساری، سمنان، بندرلنگه، حاجی‌آباد، جیرفت، شیراز، سرپل ذهاب، اصفهان، گرگان، ارومیه، کهورستان، تازیان، ایسین و سرخون) از سوش‌های محلی جمع‌آوری شد. هر جمعیت با استفاده از سیتوکروم اکسیدازیک(COI) توالی‌یابی شد. تجزیه و تحلیل واریانس مولکولی اختلاف زیادی بین جمعیت‌های مختلف *H. hebetor* نشان داد. جمعیت‌های اهواز، دهلران، جیرفت و میناب از نظر ژنتیکی بیش‌ترین اختلاف را با سایر جمعیت‌ها نشان داد. آزمون مانتل ارتباط معناداری را بین فاصله‌های ژنتیکی و جغرافیایی نشان داد ($r = 0.47, P < 0.001$). تجزیه و تحلیل فیلوژنتیک جمعیت‌ها را به دو گروه عمده تقسیم کرد (A و B). اکثر جمعیت‌ها در گروه A قرار داشتند و جمعیت‌هایی که در گروه B قرار گرفتند اکثراً از جنوب ایران بودند. براساس این پژوهش نتیجه می‌گیریم که *H. hebetor* در ایران از جمعیت‌های متنوعی تشکیل شده است. این نتایج می‌تواند به‌صورت موفقیت‌آمیز در قالب برنامه رهاسازی اشباعی به‌کار گرفته شود.

واژگان کلیدی: برنامه رهاسازی اشباعی، ساختار ژنتیکی، جدایی جغرافیایی، تنوع هاپلوتایپ، روش بایزن

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