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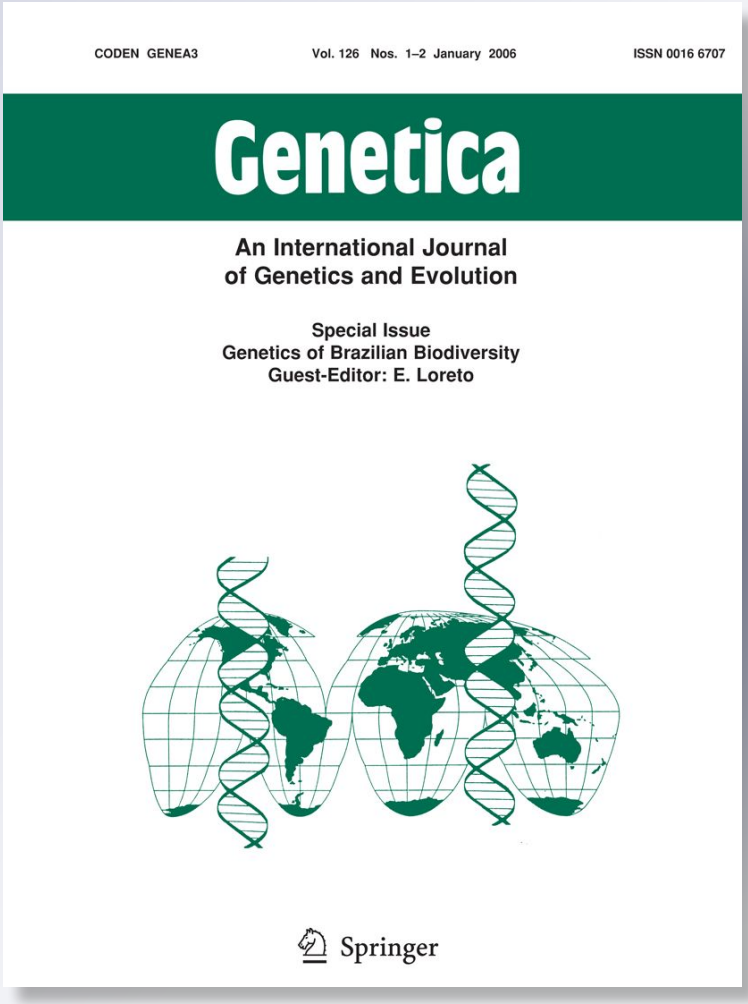

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Population genetic structure of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) based on mitochondrial cytochrome oxidase (*COI*) gene sequences

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Abstract Population genetic structure of melon fly analysed with *mitochondrial cytochrome oxidase I* gene suggested that melon fly populations across the globe is homogeneous with non-significant variation of 0.000–0.003 base substitutions per site. Test isolates representing various geographic situations across the world were placed in 26 mitochondrial haplotypes based on variations associated with a maximum of three mutational steps and the predominant haplotype i.e. H1 was present in all melon fly populations except Hawaiian population. Evolution of *mtCOI* gene suggested that the fly could have originated some 0.4 million years ago. The present study also indicated that the *B. cucurbitae* population expansion is an event of post Pleistocene warm climatic conditions with small number of founder population. The invasion of *B. cucurbitae* in Hawaii was associated with the large population size and the global presence of the fly is associated with human mediated dispersal. The very low genetic variation suggested that the fly management might be possible by large scale sterile insect techniques programme.

Keywords *Bactrocera cucurbitae* · Phylogeny · *mtCOI* · Population genetic structure · Fruit fly

C. S. Prabhakar · P. K. Mehta · P. Sood
Department of Entomology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176 062, Himachal Pradesh, India

C. S. Prabhakar (✉)
ICAR Research Complex for Eastern Region Research Centre, Plandu, Ranchi 834 010, Jharkhand, India
e-mail: csprabhakar.ento@gmail.com

P. Sood
Crop Research Sub Station, CSK Himachal Pradesh Krishi Vishvavidyalaya, Sundernagar, Mandi 174 402, Himachal Pradesh, India

S. K. Singh
Institute of Himalayan Bioresource Technology (CSIR), Palampur 176 061, Himachal Pradesh, India

P. Sharma · P. N. Sharma
Molecular Plant Pathology Laboratory, Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176 062, Himachal Pradesh, India

P. Sharma
Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India

Introduction

Bactrocera cucurbitae (Coquillett) a medium sized fly, commonly known as Melon fly, first reported by Bezzi (1913) from India is designated native of Central Asia (Virgilio et al. 2010) of Oriental region (Mwatawala et al. 2009) and more precisely India (Dhillon et al. 2005; Hu et al. 2008). At present, it is distributed all over Asia (Pakistan, Nepal, China, Sri Lanka, Taiwan, Philippines, and south-east Asian countries), Africa (Cameroon, East Africa, Egypt, Kenya, Mauritius, Tanzania), Australic-Oceania (Australia, New Guinea, Mariana and Hawaii Islands, Solomon Islands, Islands of Rota, Guam, Nauru) and South Pacific Islands of South America (Dhillon et al. 2005; Fletcher 1987; Fontem et al. 1999; Hollingsworth and Allwood 2002; Hu et al. 2008; Virgilio et al. 2010; Weems and Heppner 2001).

Bactrocera cucurbitae is a multivoltine and polyphagous fruit fly that attacks more than 125 cucurbits and solanaceous plant species (Dhillon et al. 2005; Pinero et al. 2006), grown by small and marginal farmers in the developing countries for essential dietary components and

daily livelihood. Melon fly is though one of the most studied fruit fly species in terms of its biology, distribution, quarantine, ecology, behaviour, management and control, owing to its wide distribution, highly invasive nature and importance as an agricultural pest; but still continues to cause significant damage in tropical countries ranging between 30 and 100 %, depending on the plant species and the season (Dhillon et al. 2005; Fabre et al. 2009; Mwatawala et al. 2009; Vayssieres et al. 2007, 2008). In spite of its economic importance, the detailed phylogeographic patterns and genetic structure of the species are poorly known. Consequently most of the quarantine methods and the management plans for *B. cucurbitae* are mainly based on distributional data, biology and behaviour of the pest.

The unique properties of nucleotide sequence polymorphism of mitochondrial DNA (mtDNA) can provide high resolution information on the evolutionary relations between taxonomically bound families as mitochondrial genes evolve approximately 10 times faster than single-copy nuclear DNA (Brown et al. 1979). Reasonably well conserved, maternally inherited mitochondrial haplotypes have proved robust evolutionary marker for determining intra-specific and inter-specific relationships and phylogeographical structures in various invertebrate taxa (Armstrong and Ball 2005; Avise 1994, 2000; Bermingham and Lessios 1993; Brown 1985; Brower 1994a, b; Mun et al. 2003; Nardi et al. 2005; Shi et al. 2005; Xie et al. 2006).

Very little attempt has been made to study the population structure of this pest across the world except one or two reports on molecular diversity analysis (Hu et al. 2008; Virgilio et al. 2010). Recently, Hu et al. (2008) provided first description of the East Asian *B. cucurbitae* population's phylogeography using *cytochrome oxidase I* (*COI*) sequences and observed that the genetic diversity was exceedingly low and one single haplotype was found to be predominant from Sri Lanka to China. They predicted high diversity in Indian population of *B. cucurbitae,* being the native place of this pest; however, they did not use Indian populations in their study. Whereas, Virgilio et al. (2010) studied the population structure and phylogeography of *B. cucurbitae* collected from 25 locations distributed in 20 countries including India with 13 microsatellite markers and grouped *B. cucurbitae* populations into five main groups viz. the African continent, La Reunion, Central Asia, East Asia and Hawaii. They also suggested that the higher genetic diversity in populations from Pakistan, India and Bangladesh reveals Central Asia as origin region of *B. cucurbitae* and later expanded its range to both the side of the region (Central Asia) i.e. in the east (East Asia and Hawaii) and west (Africa and the islands of the Indian Ocean) of Central Asia.

Keeping in view the importance of the pest and peculiarities of *mtCOI* gene, we made an attempt to exploit this region of the genome to study genetic structure of the melon fly prevalent in India, a country that enjoys highly variable climatic conditions from northern part approaching Himalaya to southern part touching Indian Ocean. The climatic conditions in northern part of India vary significantly in different seasons, whereas the southern part experiences almost same weather throughout the year. In addition, an attempt has also been made to utilize other available *mtCOI* gene sequences of *B. cucurbitae* from different geographical regions to structure the worldwide population and estimate the genetic diversity for better understanding of *B. cucurbitae* globally to evolve large scale management strategies like area wide management programmes for sustainable livelihood of the people from developing countries.

Materials and methods

Collection of samples

Bactrocera cucurbitae sample specimens were collected during 2009 and 2010 from 16 locations (Table 1), comprising of 8 in north western Himalayan range of India, 3 in North India where metropolitan populations resides, 3 in East India harbouring major rivers Ganga and Kosi, one in Deccan plateau (South India) and one in Nepal covering north eastern Himalayan range using insect collection net, cue lure (species specific pheromone) and by collecting fruit fly infested cucurbit fruits and flowers. The fruit fly infested samples from each location were kept in separate rearing cages ($20 \times 15 \times 18$ cm^3) under laboratory conditions at Palampur. The emerging fruit fly adults were identified on the basis of morphological descriptions given by White and Elson-Harris (1992) and Drew and Raghu (2002). Identified *B. cucurbitae* isolates were kept in separate vials and stored at −20 °C for DNA extraction. Voucher specimens are preserved in the collection of the Department of Entomology, CSKHPKV, Palampur, India.

DNA extraction

Total genomic DNA of each geographic melon fruit fly isolate was extracted from preserved specimens following the method of Prabhakar et al. (2009) with minor modifications.

PCR amplification and sequencing

Polymerase chain reaction (PCR) was performed with forward primer UEA7 5′TACAGTTGGAATAGACGTTG ATAC3′ and reverse primer UEA10 5′TCCAATGCACTA ATCTGCCATATTA3′ targeting the *mitochondrial COI*

Table 1 Sampling localities and geographic coordinates of the 5 *B. cucurbitae* populations

Sr. no.	Populations	Location(s)	Latitude DM	Longitude DM	Elevation m (amsl)	Collection date	Number of individuals (number of different haplotypes)	GenBank accession number
1	North west India	Ghumarwin	31°25′N	76°43′E	625	Aug 2009	1 (1)	HQ378206
2		Nihari	31°25′N	76°39′E	681	June 2010	1 (1)	HQ378225
3		Nadaun	31°46′N	76°20′E	460	May 2009	2 (2)	HQ378195-96
4		Indora	32°7′N	75°40′E	329	Aug 2009	2 (2)	HQ378212-13
5		Jawalamukhi	31°53′N	76°17′E	470	Aug 2009	1 (1)	HQ378215
6		Mandi	31°42′N	76°55′E	806	July 2009	2 (2)	HQ378197-98
7		Nagwain	31°49′N	77°10′E	1116	Aug 2009	1 (1)	HQ378214
8		Haroli	31°33′N	75°59′E	593	Sept 2009	1 (1)	HQ378216
9	North India	Karnal	29°41′N	76°59′E	71	Oct 2009	6 (3)	HQ378217-22
10		Delhi	28°37′N	77°9′E	229	Aug 2009	1 (1)	HQ378205
11		Ghaziabad	28°46′N	77°30′E	217	Aug 2009	1 (1)	HQ378204
12	East India	Patna	25°37′N	85°12′E	60	July 2009	4 (2)	HQ378199-202
13		Bihar Sharif	25°11′N	85°31′E	65	July 2009	1 (1)	HQ378203
14		Samastipur	25°58′N	85°40′E	58	Feb 2010	1 (1)	HQ378224
15	South India	Solapur	17°40′N	75°55′E	460	Aug 2009	5 (4)	HQ378207-11
16	Nepal	Dhankuta	26°58′N	87°20′E	1,445	July 2010	2 (2)	HQ378226-27

gene (611 bp) developed by Lunt et al. (1996). The PCR amplification was carried out in 0.2 ml PCR tubes with 20 µl reaction volume (2 µl 10× reaction buffer with 25 mM $MgCl_2$ (Life Technologies India, Pvt. Ltd), 1 µl 20 pmol of each primer, 0.5 µl 10 mM $dNTP_S$ (Fermentas), 0.2 µl 5 U *Taq* polymerase (Life Technologies (India), Pvt. Ltd) and 2 µl 10 ng of DNA template). Amplifications were performed in GeneAmp 9700 thermal cycler (Applied Biosystems, USA) with an initial denaturation step of 3 min at 94 °C followed by 35 cycles at 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min and a final elongation step at 72 °C for 30 min. The PCR product was separated in 2 % (w/v) agarose gel using TAE buffer (40 mM Tris–acetate, 1 mM EDTA). Amplified PCR products (~611 bp) using *mtCOI* gene specific primers were freeze dried (CHRIST *ALPHA I-2LD*) and custom sequenced (ABI PRISM 310^{TM} Genetic Analyzer, Applied Biosystems, USA) using same upstream and downstream primers (Xcelris Labs Limited, India).

Nucleotide sequence analysis

All 70 obtained sequences of *B. cucurbitae* (32 individual sequences from 5 populations and 38 sequences collected from GenBank, NCBI (Table 2) from 5 populations) were aligned using ClustalW as implemented in MEGA 4.1

Table 2 List of *mtCOI* gene sequences of *Bactrocera cucurbitae* obtained from GenBank database (NCBI)

Sr. no.	Country	Location	Accession no.	Number of individuals (number of different haplotypes)
1	China	Guangdong	AY398758	1 (1)
2	China	–[a]	EU048559- EU048568	10 (6)
3	China	–[a]	EU599634	1 (1)
4	Japan	Amami-Oshima island, Kagoshima	AB192449	1 (1)
5	Japan	Ishigaki, Okinawa	AB192450	1 (1)
6	Japan	Yaeyama islands	AY530900	1 (1)
7	Sri Lanka	Puttalam	AB192451	1 (1)
8	Thailand	Chiang Mai	AB192452	1 (1)
9	Thailand	–[a]	AF423110	1 (1)
10	USA	Hilo, Hawaii	AY945033-AY945052	20 (5)

[a] Location not known

(Tamura et al. 2007) and 32 sequences sequenced in this study deposited in GenBank, NCBI (accession numbers HQ378195-HQ378222 and HQ378224-HQ378227). A minimum spanning network among *B. cucurbitae* haplotypes was constructed using 95 % parsimony criteria with the programme TCS 1.21 (Clement et al. 2000). Haplotypes numbers and distribution, polymorphic sites and nucleotide diversity were assessed by ARLEQUIN 3.11 (Excoffier et al. 2005). Analysis of molecular variance (AMOVA) was performed to assess the genetic structure and genetic variability within and between the populations of *B. cucurbitae*. AMOVA analyses worked by partitioning the total variations between and within populations of *B. cucurbitae* and were run using the ARLEQUIN 3.11 (Excoffier et al. 2005). A two-part AMOVA analysis was performed by testing the genetic structure as a factor of variation among individuals within a population and between populations. Pair-wise comparisons were conducted to test genetic divergence (F_{st}) using the ARLEQUIN 3.11.

Analysis of Genetic and Phylogenetic relationships was performed in MEGA 4.1 (Tamura et al. 2007) keeping *Bactrocera tau*, *B. scutellaris*, *Musca domestica* and *Locusta migratoria* as out groups. Genetic distances among geographical population and outgroups were calculated based on pairwise matrix of sequence divergences using Kimura two-parameter method. The neighbour-joining (Saitou and Nei 1987) method was used for phylogeny reconstruction. Confidence levels for (Felsenstein 1985) NJ tree were assessed by bootstrap (1,000 replications).

Results

The 32 sequences generated in the present study were merged with 38 NCBI GenBank sequences to obtain a final alignment of 70 sequences. After sequence alignment, 31 polymorphic positions were observed in a span of 575 bp of the aligned sequences (5.3 % variation). All the individuals sequenced in this study were 100 % (575) overlapped with other sequences of *B. cucurbitae* collected from GenBank (NCBI). All 31 polymorphic positions were characterised by 18 singletons, 5 parsimony informative and 8 sites recorded as indels (insertions or deletions). Eighteen transitions and 5 transversions were also observed. An overall transition/transversion ratio was found to be 3.6 with mean genetic distance of 0.000–0.003 between *B. cucurbitae* populations However, among different species it ranged from 0.051 to 0.053, 0.155–0.157, 0.212–0.215 and 0.923–0.929 between *B. cucurbitae*/*B. tau*, *B. cucurbitae*/*B. scutellaris*, *B. cucurbitae*/*Musca domestica* and *B. cucurbitae*/*Locusta migratoria*, respectively (Table 3). The phylogenetic tree representing various test isolates (Fig. 1) revealed that, *B. cucurbitae* occupied a closer position to *B. tau* (with 97 % bootstrap support), whereas other out group species were separated as per their rank. The analysis of molecular variance showed highest variance to be present within populations (63.96 %, $P < 0.001$) than among the populations (36.04 %, $P < 0.001$). The highest pairwise fixation index (0.75497) (Table 4) was observed for the Hawaii and East

Table 3 Mean genetic distances between populations of *B. cucurbitae* and outgroups

Sr. no.	Populations	Mean pairwise genetic distance													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Hawaii		0.000	0.000	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.009	0.016	0.020	0.073
2	North west India	0.001		0.000	0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.009	0.016	0.020	0.074
3	East India	0.000	0.000		0.001	0.001	0.001	0.000	0.000	0.001	0.000	0.009	0.016	0.020	0.073
4	North India	0.002	0.002	0.002		0.001	0.001	0.001	0.001	0.001	0.001	0.009	0.016	0.020	0.074
5	South India	0.002	0.003	0.002	0.003		0.001	0.001	0.001	0.001	0.001	0.009	0.016	0.020	0.074
6	Nepal	0.001	0.001	0.001	0.002	0.003		0.001	0.001	0.001	0.001	0.009	0.016	0.020	0.073
7	Sri Lanka	0.000	0.000	0.000	0.002	0.002	0.001		0.000	0.001	0.000	0.009	0.016	0.020	0.073
8	Japan	0.000	0.000	0.000	0.002	0.002	0.001	0.000		0.001	0.000	0.009	0.016	0.020	0.073
9	Thailand	0.001	0.001	0.001	0.002	0.003	0.002	0.001	0.001		0.001	0.010	0.016	0.020	0.073
10	China	0.001	0.002	0.001	0.003	0.003	0.002	0.001	0.001	0.002		0.009	0.016	0.020	0.074
11	*B. tau*	0.053	0.053	0.052	0.052	0.051	0.051	0.052	0.052	0.053	0.053		0.016	0.019	0.075
12	*B scutellaris*	0.156	0.156	0.156	0.156	0.157	0.155	0.156	0.156	0.155	0.156	0.145		0.022	0.078
13	*Musca domestica*	0.213	0.213	0.213	0.214	0.215	0.212	0.213	0.213	0.214	0.214	0.206	0.232		0.078
14	*Locusta migratoria*	0.923	0.924	0.923	0.927	0.929	0.923	0.923	0.923	0.923	0.925	0.917	0.954	0.967	

All results are based on the pairwise analysis of 74 sequences

Mean K2P distances are below diagonal and Standard error estimate(s) are above diagonal were obtained by a bootstrap procedure (1,000 replicates

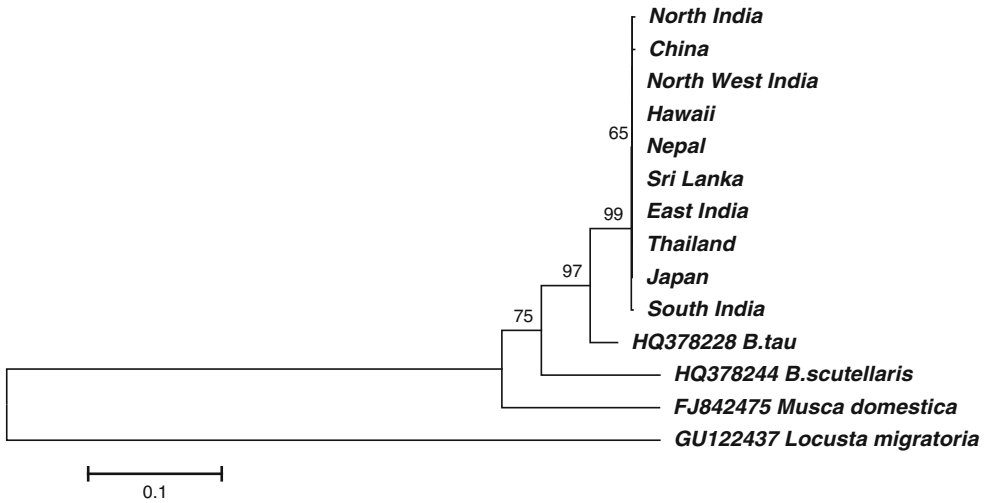

Fig. 1 Phylogenetic tree based on *mtCOI gene* of 10 *B. cucurbitae* populations with *B. tau, B. scutellaris, Musca domestica and Locusta migratoria* as outgroups using the neighbor-joining method and confidence level was calculated with the bootstrap test (1,000 replicates)

Table 4 Population specific, overall and pairwise fixation index (F_{st}) of *B. cucurbitae*

Population	Hawaii	North west India	East India	North India	South India	Nepal	Sri Lanka	Japan	Thailand	China
Hawaii (0.41365)[a]	0.00									
North west India (0.30540)[a]	0.58059	0.00								
East India (0.45889)[a]	0.75497	–	0.00							
North India (0.29147)[a]	0.60671	0.04488	0.03614	0.00						
South India (0.28746)[a]	0.68657	0.16606	0.29412	–	0.00					
Nepal (0.33984)[a]	0.74166	0.09142	0.53846	0.08522	0.15441	0.00				
Sri Lanka (0.45889)[a]	0.66667	–	–	–	–	–	0.00			
Japan (0.45889)[a]	0.72215	–	–	–	0.11765	0.25		0.00		
Thailand (0.39936)[a]	0.72642	–	0.53846	–	0.08686	–	–	0.25	0.00	
China (0.31338)[a]	0.57787	0.0133	–	0.02425	0.16066	0.09774	–	–	–	0.00
OVERALL F_{st}	0.36042									

[a] Population specific fixation index

India population while it was minimum for China and North west India (0.0133). The overall fixation index observed for all populations was "0.36042". Population specific fixation index was found highest for Japan, Sri Lanka and East India populations (0.45889) while it was minimum for South India (0.28746) population.

On the basis of sequences variation, twenty-six sequence variants (haplotypes) were observed in the ten *B. cucurbitae* populations, corresponding to 70 *mtCOI* gene sequences (Table 5). Only four haplotypes were shared by at least two individuals and 22 haplotypes were unique. Haplotype H1 was the predominant haplotype shared by all *B. cucurbitae* populations except South India and Hawaii and occupied a central position in the minimum spanning network (Fig. 2). Among other shared haplotypes, H9 was shared by two populations (North India and South India), whereas H4 and H22 were represented by two or more individuals of a single population i.e. North west India and Hawaii, respectively (Table 5).

Discussion

Bactrocera cucurbitae isolates from diverse geographical regions used in the present study exhibited very low genetic diversity ranging between 0.000 and 0.003. Hu et al. (2008) also reported almost similar pattern between different *B. cucurbitae* isolates collected from China as

Table 5 Distribution and frequency of different mitochondrial haplotypes of *B. cucurbitae* in different populations

Populations	Mitochondrial haplotypes													
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14
North west India	6	1	1	2	1									
East India	6													
North India	4					1	1	1	1					
South India	0								2	1	1	1		
Nepal	1												1	
China	5													1
Japan	3													
Sri Lanka	1													
Thailand	1													
Hawaii														
Overall frequency	0.38	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.04	0.01	0.01	0.01	0.01	0.01

Populations	Mitochondrial haplotypes												
	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25	H26	*n*
North west India													11
East India													6
North India													8
South India													5
Nepal													2
China	1	1	1	1	1	1							12
Japan													3
Sri Lanka													1
Thailand							1						2
Hawaii								16	1	1	1	1	20
Overall frequency	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.22	0.01	0.01	0.01	0.01	70

n indicates the number of sequences used in a population

well as south-east Asia. Hu et al. (2008) observed that *B. cucurbitae* populations present in China and south-east Asia did not possess significant variations. This study also supports the fact that *B. cucurbitae* populations are homogeneous irrespective of their geographical distribution as evident from very low intra-specific pair wise genetic distance. Contrary to this high intra-specific distances have also been reported for the other fruit flies (Mun et al. 2003; Nardi et al. 2005; Ochando and Reyes 2000; Shi et al. 2005, 2010).

Based on studies of insect mtDNA, Brower (1994b) suggested that the molecular clock could be calibrated to 2.3 % pairwise sequence divergence per million years. Using this value, *B. cucurbitae* could have originated some 0.4 million years ago, exhibiting its recent evolution in nature. Using the same estimate of Brower (1994b), Jamnongluk et al. (2003) suggested that the *B. tau* and *B. dorsalis* species complex could have arisen some 5 and 15 million years ago, respectively. This *B. tau* species is similar in morphology and host range like *B. cucurbitae*. High genetic uniformity in the present studies also suggested the recent origin of *B. cucurbitae* as compared to *B. tau* and *B. dorsalis* and the saturation of nucleotide sequences might not have occurred to form the cryptic species or origin of complex as in case of *B. tau* (Jamnongluk et al. 2003).

Out of 26 haplotypes consisting of 70 *B. cucurbitae* sequences analysed in the present study, 13 (50 %) haplotypes were from Indian subcontinent and rest 13 were distributed in their respective geographical population localities. Therefore, the haplotypes diversity among *B. cucurbitae* population is more in Indian subcontinent than any other region under study. Recently, Hu et al. (2008) published population genetic structure of *B. cucurbitae* from China and south-east Asia with the analysis of 64 individuals from eight geographically distinct populations excluding Indian population. They reported 8 haplotypes (12.50 %) from 64 individuals. Whereas in this study, 13 haplotypes (40.62 %) was observed in 32 individuals distributed in five populations of Indian subcontinent. Thereby,

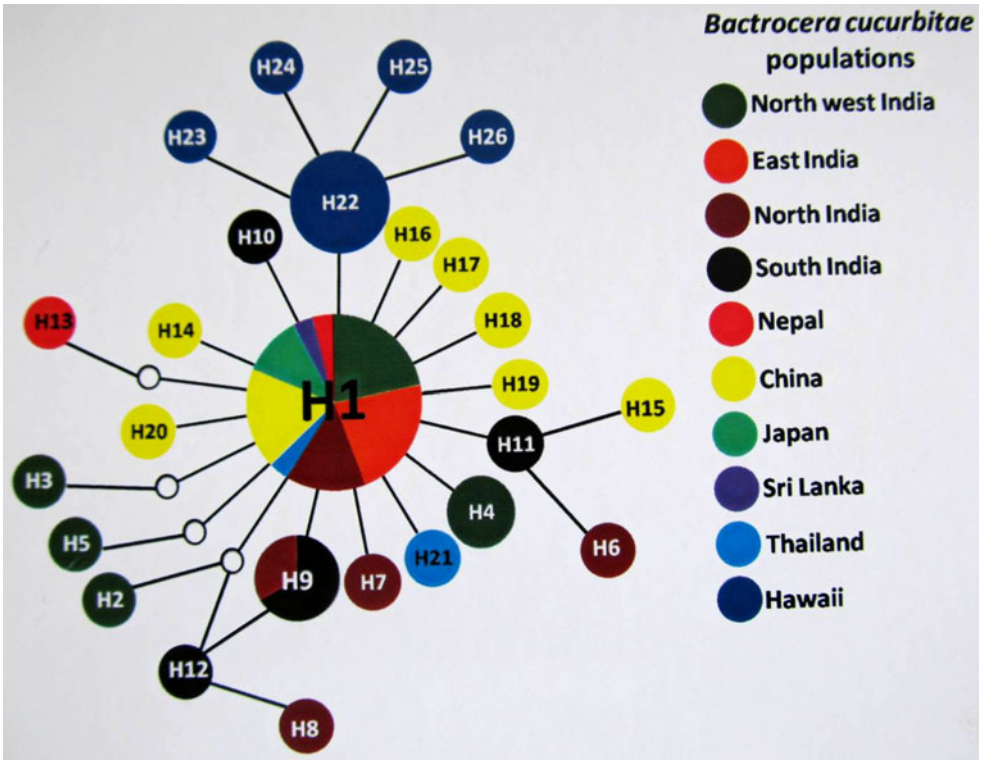

Fig. 2 Minimum Spanning Network of the 26 mitochondrial haplotypes, observed in a set of 70 individuals from all 10 *Bactrocera cucurbitae* populations. Area of circles is proportional to haplotype frequency in the dataset. Geographical region of each haplotype is colour coded. One, two and three mutational steps are shown. *H* Haplotype (H1–H26). (Color figure online)

suggesting that the haplotypes diversity is more among Indian subcontinent than that of China and south-east Asia. The present findings are also supported by the results of Virgilio et al. (2010), who studied phylogeography and genetic structure of 25 populations of *B. cucurbitae* collected from different countries using 13 microsatellite loci and suggested that populations are more diverse in central Asia (India, Pakistan and Bangladesh) than any other populations of the world. Their study provides supportive evidence about the central Asia as centre of origin of *B. cucurbitae*. The present finding on haplotype diversity in *B. cucurbitae* population in Indian subcontinent also propounds the possible origin of *B. cucurbitae* from central Asia and more precisely in India. Moreover, as the present study is based upon the variations present in mtDNA sequence which have additive advantages over microsatellites that the mitochondrial genome is exclusively maternally inherited, haploid, and does not undergo recombination thus the individuals from one matrilineal are assumed to share one mtDNA haplotype. The mtDNA (*mtCOI* gene) provides a highly informative tool for matrilineal relationship studies within breeds to detect their differentiation and to refer to different founder populations. Although, H1 haplotype occupied central position in the minimum spanning network, connected by few mutations to other haplotypes (Fig. 2), indicates recent population expansion from a limited number of founders followed by population growth as coalescence theory predicts (Avise 2000; Slatkin and Hudson 1991) in case of recent invasion. Nevertheless, this fly is originated in Indian subcontinent and endemic to this region and not a recent invader (Dhillon et al. 2005; Hu et al. 2008). However, high genetic uniformity and low genetic variability in *B. cucurbitae* populations in the region from where the fly have originated, suggested post-Pleistocene population expansion with limited number of founders and population expansion thereupon. In support of these findings, the distribution for *B. cucurbitae* was strongly biased toward low divergence values with a mode of three nucleotide changes, suggesting a relatively recent expansion event with post-Pleistocene warming and with the domestication and range expansion of several cucurbit hosts. Present phylogeographic patterns are also supporting the post Pleistocene population expansion of the fly when haplotype frequency distributions were strongly skewed with 50 % of the haplotypes present only in a single region (Indian subcontinent). Analysis of molecular variance also revealed that the most of variation was within populations with no significant genetic structuring in the populations fit the model of sudden population expansion as predominant haplotypes occupy a central position in the network (Fig. 2). This kind of post Pleistocene population expansion has been documented in relatively few taxa such as in human (Eshleman et al. 2004; Tanaka et al. 2004), pea aphid (Peccoud et al. 2009) and bat (Bilgin et al. 2008).

With the hypothesis of Indian origin of the *B. cucurbitae* with recent expansion to the rest of the world, it still remains unclear that why none of the Hawaiian haplotypes identified in this study was present in Indian subcontinent. The pairwise fixation index (Table 4) also showed that the Hawaiian population has least genetic similarity as compared to other populations. One explanation could be that as the haplotypic variation of the *B. cucurbitae* population in Indian subcontinent is high, the entire diversity of the species on this subcontinent was only partially sampled. The first introduction of *B. cucurbitae* into the Hawaii was in 1895 (Bess et al. 1961) and recent in nature. However, the five haplotypes detected in Hawaiian population advocates the invasion of *B. cucurbitae* with its large population or multiple invasions. The variation in Hawaiian population was also observed by Virgilio et al. (2010) with microsatellite markers and they suggested that the adventives population must have been relatively large and polymorphic as the invasion of Hawaii was not associated to a bottleneck. The high homogeneity of *mtCOI* gene sequences between individuals originating in the Indian subcontinent and other countries alongwith low fixation index in turn suggest a common origin of the *B. cucurbitae* infesting these regions.

The similarity of *B. cucurbitae* population of India with that of Japan, where it has been eradicated with SIT (sterile insect technique) programme (Koyama et al. 2004), make this fly a suitable candidate for its management through SIT in India too. This also suggests that any large scale *B. cucurbitae* management programme may work in all populations including India as the genetic makeup of the populations is same, which is also revealed by F_{st} values (Table 4). However, the success of large scale (international/inter-continental) SIT programme depends on the mating compatibility of the released laboratory strain with the wild flies. To support the above phenomenon, mating tests among three *B. cucurbitae* populations from Mauritius, Seychelles and Hawaii (genetic sexing strain) were conducted by Sookar et al. (2010) and they observed that the sexual activity among the three melon fly populations was similar as non significant non-random, assortative mating was observed. Therefore, they concluded that melon flies from Mauritius, Seychelles and the Hawaii are compatible, at least under semi-natural conditions. The compatibility of Hawaii population with other geographically isolated population is in agreement with the high genetic similarity observed in the present study between India and Hawaii (USA) melon fly population. This also suggested that the *B. cucurbitae* invasion in different countries is recent in nature; most possible source might be India (origin place) and presence of *B. cucurbitae* in Asian countries as well as Hawaii Island of USA showed no historical separation as they are coming from geographically distinct places but forming single clade. However, large number of samples from different locations of Indian subcontinent is needed because of diverse climatic conditions prevailing in the subcontinent and centre of origin of the melon fly. In earlier work, only three locations were analysed by different worker from the world. Any international fruit fly eradication programme can use SIT technique with the help of sterile strain of *B. cucurbitae* used in Japan for the eradication of this dreaded pest for sustainable vegetable production and better livelihood of the farming community. However, before that large number of samples from different locations needs to be analysed for identification of any strain or species evolution due to point mutation accumulation. This would also save the environment as well as the farming community and consumers from health hazards due to the indiscriminate use of pesticide.

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