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Population genetic structure of the pumpkin fruit fly, *Bactrocera tau* (Walker) (Diptera: Tephritidae) in Himachal Pradesh, India

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ABSTRACT

The population genetic structure of the pumpkin fruit fly, *Bactrocera tau*, a fruit fly pest that causes significant losses to cucurbit cultivations, has been studied in Himachal Pradesh (India) using mitochondrial cytochrome oxidase I (mtCOI) gene sequences. Levels of differentiation (genetic distances and F_{ST} values) among samples from different locations are minimal, suggesting the local occurrence of a large and geographically undifferentiated population, with the possible exception of population Solan. Nevertheless, overall genetic variability is substantial, with 10 different haplotypes detected in 16 individuals and only one of these – likely the original one as it occupies a central position in the network and is found at a relatively high frequency –shared between multiple populations. The phylogenetic analysis of local *B. tau* samples in the context of the different sibling species that constitute the *B. tau* complex in its South-East Asia region of origin revealed that local *B. tau* is closely related to *B. tau* species *A* from Thailand. This should be taken into account in any intervention aimed at the control of this pest, e.g. area wide integrated pest management (AW IPM). The marked local genetic uniformity and predominance of one single species of the species complex further suggest that the sterile insect technique (SIT) may be a viable option.

1. Introduction

The pumpkin fruit fly, *Bactrocera tau* (Walker), is widely distributed throughout South Asia (India, Sri Lanka, Bangladesh and Bhutan), South East Asia (Thailand, Malaysia, Vietnam, Philippines and Indonesia) and East Asia (Taiwan and South China) (White and Elson-Harris, 1992; Drew and Roming, 1997; Prabhakar et al., 2012a). Being multivoltine and highly polyphagous, *B. tau* can attack more than 50 cultivated as well as wild plant species from families Anacardiaceae, Cucurbitaceae, Elaeocarpaceae, Moraceae, Myrtaceae, Oxaalidaceae, Rutaceae, Sapotaceae and Solanaceae (Allwood et al., 1999; Huque, 2006). Furthermore, the species infests a wide range of commercially important cucurbit crops such as cucumber, luffa, pumpkin,

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melon, bitter gourd, bottle gourd, ribbed gourd, sponge gourd, ash gourd, snake gourd, sweet gourd and summer squash (Drew and Roming, 1997; Prabhakar et al., 2012a).

B. tau is widely distributed in different regions of India, but its economic damage to cucurbit crops is more severe in the North-Western and North-Eastern hilly regions of the Indian Himalaya (Borah and Dutta, 1996; Prabhakar et al., 2009a, 2012a). Himachal Pradesh, one of the major vegetable growing states of India, suffers significant economic losses that may account for 80% of total cucurbit crops (Prabhakar et al., 2009a; Sood et al., 2010). The majority (96%) of the state is characterized by hills and/or higher mountain ranges, with *B. tau* activity being more significant in areas characterized by low to mid hills and absent in high hill regions (>3200 m amsl).

Based on recent works by Baimai et al. (2000), Jamnongluk et al. (2003) and Thanaphum and Thaenkham (2003), it is now well established that *B. tau* is not to be regarded as a single species, but rather a complex of different sibling species which have been tentatively systematized into eight forms designated as *B. tau* sp *A, B, C, D, E, F, G* and *I* based on specific host-plant preferences, cytological differences, external morphology, allozyme electrophoretic studies, molecular genetic markers and mtCOI gene sequences. This, in turn, led to the recognition that more specific and niche based management programmes are needed for an effective control of this pest (Thanaphum and Thaenkham, 2003).

Morphological differences are often unable to provide adequate resolution to determine the status of species, prompting for the implementation of alternative methods, such as DNA barcoding, to identify and characterize the different species/forms. Due to its pattern of maternal inheritance, absence of recombination and rate of evolution approximately 10 times faster than single-copy nuclear DNA (Brown et al., 1979), the mtCOI is appropriate to characterize taxonomically similar entities and is widely used as a DNA barcode marker (Hebert et al., 2003). Furthermore, mtCOI sequences, and mitochondrial haplotypes in general, have proved to be robust evolutionary markers for determining intra- and inter-specific relationships and phylogeographic structures in various invertebrate taxa, including fruit flies (Armstrong and Ball, 2005; Avise, 2000; Nardi et al., 2005; Prabhakar et al., 2012b).

Aim of the present study is to investigate the genetic variability and population structure in populations of *B. tau* from the Himachal Pradesh (India) and to identify their relationships in the context of the different species that constitute the *B. tau* species complex.

2. Materials and methods

2.1. Collection of infested fruits and rearing of fruit flies

Fruit fly infested cucurbit fruits and flowers were collected during years 2008–2010 from 12 locations in six districts of Himachal Pradesh (Table 1). Samples from each location were kept in separate rearing cages (20 × 15 × 18 cm) under laboratory conditions (Temp. 25±2 °C and RH 75–80%) at Palampur, India (Latitude: 32°6′N, Longitude: 76°3′E, Altitude: 1290 m amsl). Emerging fruit fly adults were identified based on the morphological descriptions given by White and Elson-Harris (1992) and Drew and Raghu (2002). Identified *B. tau* specimens were stored at −20 °C until DNA extraction, and voucher specimens are preserved in the collection of the Department of Entomology, CSK HPKV, Palampur, India.

2.2. DNA extraction

Total genomic DNA was extracted from single *B. tau* specimens following the method of Prabhakar et al. (2009b), with minor modifications. Samples were frozen in liquid nitrogen for 1 min, ground to a fine powder using a micro-pestle,

Table 1
Sampling locations and geographic coordinates of the six *Bactrocera tau* populations from Himachal Pradesh, India.

Sr. no.	Populations	Location(s)	Latitude	Longitude	Elevation m (amsl)	Collection date	Host plant/cue lure	*n*	GenBank accession number
1	Bilaspur	Ghumarwin	31°25′ N	76°43′ E	625	Aug 2009	*Cucumis sativus* Linnaeus	1	HQ378235
2		Chandpur	31°21′ N	76°47′ E	1020	Aug 2009	*Cucumis sativus* Linnaeus	1	HQ378241
3		Nihari	31°25′ N	76°39′ E	681	Sep 2009	Cue lure	1	HQ378243
4	Chamba	Banikhet	32°33′ N	75°57′ E	1538	Aug 2008	*Cucumis sativus* Linnaeus	1	HQ378232
5	Hamirpur	Nadaun	31°46′ N	76°20′ E	460	May 2009	*Lagenaria siceraria* (Molina)	1	HQ378228
6		Nadaun	31°46′ N	76°20′ E	460	May 2009	*Momordica charantia* Linnaeus	1	HQ378229
7		Nadaun	31°46′ N	76°20′ E	460	May 2009	*Cucumis sativus* Linnaeus	1	HQ378233
8	Kangra	Palampur	32°6′ N	76°32′ E	1290	Jun 2009	*Cucumis sativus* Linnaeus	1	HQ378230
9		Palampur	32°6′ N	76°32′ E	1290	Aug 2009	*Momordica charantia* Linnaeus	1	HQ378237
10		Jawalamukhi	31°53′ N	76°17′ E	470	Aug 2009	*Cucumis sativus* Linnaeus	1	HQ378239
11		Jawalamukhi	31°53′ N	76°17′ E	470	Aug 2009	*Momordica charantia* Linnaeus	1	HQ378240
12		Shahpur	32°13′ N	76°11′ E	912	May 2010	*Momordica charantia* Linnaeus	1	HQ378242
13	Mandi	Mandi	31°42′ N	76°55′ E	806	Aug 2009	*Cucumis sativus* Linnaeus	1	HQ378234
14		Barot	32°02′ N	76°50′ E	2690	Aug 2009	*Cucurbita maxima* Duchesne	1	HQ378238
15		Nagwain	31°49′ N	77°10′ E	1116	Aug 2009	*Momordica charantia* Linnaeus	1	HQ378236
16	Solan	Nauni	30°56′ N	77°20′ E	1546	Jul 2009	*Cucurbita pepo* Linnaeus	1	HQ378231

n Number of individuals of *B. tau* sequenced.