

Genetic and geometric analyses of *Zeugodacus tau* (Walker) (Diptera: Tephritidae) in different host types

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ABSTRACT

Zeugodacus tau (Walker), a fruit fly pest, has caused damage to fruits and cucurbit crops in Southeast Asia including Thailand. Different cucurbit fruits have some unique characteristics that directly or indirectly influenced the fruit fly pest. To investigate either adaptation or specification of *Z. tau* to different fruit types, genetic and geometric analyses were used for verification. The Cytochrome Oxidase I haplotypes of *Z. tau* collected from three cucurbit fruit species, luffa (*Luffa aegyptiaca*), bitter gourd (*Momordica charantia*) and winter melon (*Benincasa hispida*) were examined by Single Strand Conformation Polymorphism analysis. Nine distinct haplotypes (A, B, C, D, E, F, G, H and I) were found. The unique haplotypes were detected in *Z. tau* population from luffa (haplotypes E, F and H) at very low levels, while haplotypes C and D were presented in all populations at higher levels. Moreover, mean haplotype diversities were high for all populations expected to relate with gene flow among populations. The analysis of molecular variance (AMOVA) also revealed low F_{ST} value (0.085) of the genetic differences among *Z. tau* populations from different fruit types suggesting that *Z. tau* can move freely among host plants. In contrast, the morphological differences in *Z. tau* associated with host fruit species were investigated by geometric analysis based on 13 wing landmarks. The differences were described by two discriminant factors (male: DF1 = 53% and DF2 = 47%, female: DF1 = 64% and DF2 = 36%). The differences in wing morphology of *Z. tau* from each population indicated that fruit fly species play an important role during larval adaptation of phytophagous insects.

Keywords: cucurbit fruits, *Zeugodacus tau*, adaptation

INTRODUCTION

Fruit fly is an important pest problem worldwide. The most important pest is true fruit fly, family Tephritidae (Diptera), that include more than

5,000 species from 500 genera among six subfamilies (Uchôa, 2012). *Zeugodacus* spp. is one of the biggest genus whose host fruits are cucurbit fruits. The well-known species include *Zeugodacus cucurbitae* and *Zeugodacus tau*. Both species infest many fruit types and use fruits as the food source for their larvae. However, *Z. tau* is a polyphagous insect that is able to infest a wider range of fruit types than *Z. cucurbitae* (Areekul, 1986). Therefore, *Z. tau* is the more destructive species.

Zeugodacus tau (Walker) is one of the pests that cause serious problems throughout Southeast Asia including Thailand (White and Elson-Harris, 1992; Yong *et al.*, 2017). *Z. tau*'s larvae are fed and developed inside many host plants, particularly family Cucurbitaceae (Christenson and Foote, 1960; Singh *et al.*, 2010), most of which are edible and important in economic such as bitter gourds (*Momordica charantia*), cucumbers (*Cucumis sativus*), winter melons (*Benincasa hispida*), pumpkins (*Cucurbita moschata*), etc. Many studies have shown that the adaptation and specification of phytophagous insects are related to host plant characteristics (Bush, 1969; Kitthawee and Dujardin, 2010; Fontaine and Thébault, 2015; Shi *et al.*, 2017). Thus, the different characteristics of cucurbit fruits could directly or indirectly influence *Z. tau* variation.

The aims of this study were to characterize the pattern of genetics and morphology of *Z. tau* from different fruit types and to evaluate either adaptation or specification of *Z. tau* to their host fruit species. The results will provide the knowledge for better understanding of adaptation and/or specification and for develop pest control strategies.

MATERIALS AND METHODS

Sample collections

Z. tau samples were collected from infested luffa (*Luffa aegyptiaca*), bitter gourd (*Momordica charantia*) and winter melon (*Benincasa hispida*) in Loei province, Thailand. Fruits with oviposit marks

were collected and kept in insectaria (Department of Biology, Faculty of Science, Mahidol University) with codes indicating host plants. Fruit fly larvae were reared until they became adult. The adult *Z. tau* samples were classified out of relative species by morphology (White and Elson-Harris, 1992; Baimai *et al.*, 2000) and preserved in 70% EtOH for further experiments.

Molecular analysis

DNA was extracted from head and thorax of *Z. tau* by phenol chloroform method (Cockburn and Fritz, 1996), and was amplified in mitochondrial Cytochrome Oxidase I (mtCOI) region by forward primer: 5' TGG AGA CGA CCA AAT CTA 3' and reverse primer: 5' GAG GAA ATA CCA GCT AAA TG 3' (Somsill *et al.*, 2015). The PCR products which had enough quantity were subsequently analyzed by single strand conformation polymorphism (SSCP). Each prepared product was placed in a well of polyacrylamide gel and differentiated for each single strand by electricity. The polyacrylamide gel was dyed

with silver nitrate solution and showed haplotype patterns. Haplotypes were scored and calculated for haplotype frequency and haplotype diversity. A population pairwise analysis and analysis of molecular variance (AMOVA) were calculated in Arlequin program (Excoffier and Lischer, 2010) to investigate the population genetic differentiation.

Geometric analysis

The left and right wings of *Z. tau* (the same set of the molecular analysis) were mounted in glass slides by Hoyer fluid, covered with cover slips and incubated overnight at 65°C. Wings were photographed under stereomicroscope connected to a digital camera. Thirteen landmarks (Figure 1) were assigned and digitized on forewing venations of each specimen using Collecting Landmark for Identification and Characterization (CLIC) software developed by Dujardin J. P., available at <http://xyom-clic.eu>. Data collections and analyses were performed through the various modules in the CLIC software.

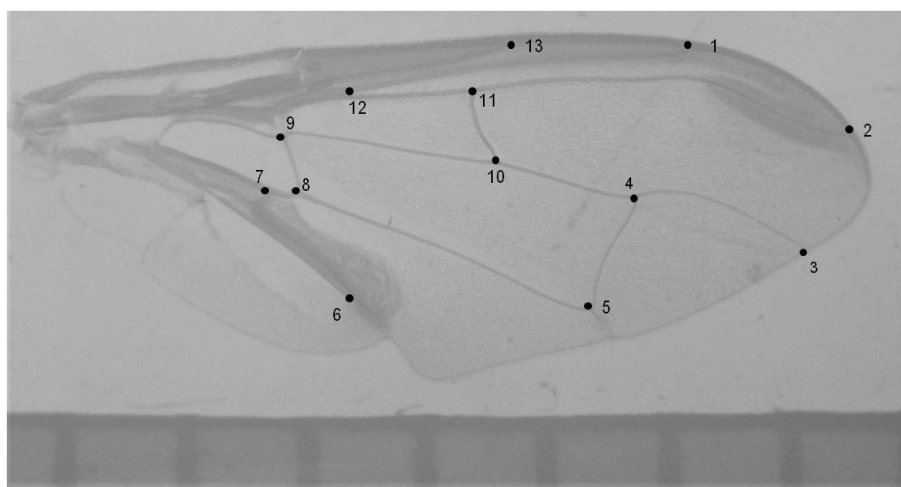


Figure 1 Fore wing of *Z. tau* showing 13 landmarks used in the geometric analysis.

RESULTS AND DISCUSSION

The mtCOI gene data were obtained from 97 individuals of *Z. tau* collected from three host plant species. A total of nine distinct haplotypes (A, B, C, D, E, F, G, H and I; Figure 2), consisting of six shared and three unique haplotypes, were identified (Table 1). Haplotypes A, B, C, D, G and I were shared among at least two populations. The most common shared haplotypes C (24.70%) and D (24.70%) were found in all individuals tested. The other shared haplotypes were found to be less than 15% of the samples. The haplotypes E, F and H were unique in samples from

luffa. Although unique haplotypes suggested the genetic variation between populations from different host plants, the degree of these haplotype frequencies were at low levels ($E = 5.20\%$, $F = 3.10\%$ and $H = 1.00\%$). These low levels of unique haplotypes could reflect a low genetic differentiation between *Z. tau* populations from different fruit types. The genetic variation was examined and confirmed by pairwise F_{ST} and AMOVA analyses (Table 2). Pairwise analysis showed that *Z. tau* population from bitter gourd was significantly different ($p < 0.05$) from luffa and winter melon populations with low F_{ST} values

(0.087 and 0.109). The pairwise F_{ST} between *Z. tau* populations from luffa and winter melon was also low (0.058) indicating low genetic differentiation or high level of gene flow between populations. For genetic analysis by AMOVA (Table 2), 8.51% of the total variation occurred among populations while 91.49% occurred within populations. These results implied a small barrier to gene flow among populations.

The genetic variation within *Z. tau* population was high. This information indicated that gene flow can potentially reduce genetic differentiation and be responsible for high intra-population variations as occurred in several reports (Kunprom *et al.*, 2015). Although AMOVA analysis demonstrated a significant degree ($p < 0.05$) of genetic differentiation among

populations, the F_{ST} value (0.085) showed that those differentiations among *Z. tau* populations were weak. This result implied that dispersal may be unlimited among host plants. This interpretation was further supported by the results of SSCP haplotype diversities (Table 1). Mean haplotype diversities were high for populations from luffa, bitter gourd and winter melon (0.843, 0.770 and 0.775 respectively). High haplotype diversity is expected to relate with a widespread of insect species (Choudhary *et al.*, 2016). It is possible that *Z. tau* populations from these fruit types still receive sufficient gene flow from other populations because *Z. tau* is the most common cucurbit fruit flies in several regions of the world (Yong *et al.*, 2017; Boopathi *et al.*, 2017).

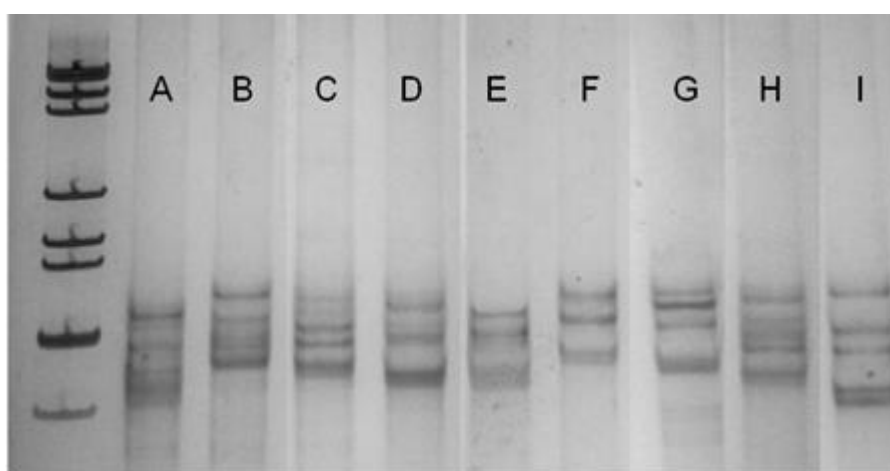


Figure 2 Haplotype patterns of COI gene in *Z. tau* obtained from SSCP technique. The letter in each lane represents different haplotypes.

Table 1 Sample size (n), haplotype frequency and haplotype diversity (Hd) of *Z. tau* from different fruit types

Fruit types	n	COI haplotype frequency (n)									Hd
											Mean
		A	B	C	D	E	F	G	H	I	(SD)
Luffa				0.290	0.194	0.161	0.097	0.097	0.032	0.129	0.843
<i>L. aegyptiaca</i>	31			(9)	(6)	(5)	(3)	(3)	(1)	(4)	(0.033)
Bitter gourd		0.032	0.226	0.097	0.258			0.355		0.032	0.770
<i>M. charantia</i>	31	(1)	(7)	(3)	(8)			(11)		(1)	(0.040)
Winter melon		0.229	0.142	0.343	0.286						0.750
<i>B. hispida</i>	35	(8)	(5)	(12)	(10)						(0.029)
		0.093	0.124	0.247	0.247	0.052	0.031	0.144	0.010	0.052	0.835
Total	97	(9)	(12)	(24)	(24)	(5)	(3)	(14)	(1)	(5)	(0.017)

Table 2 Pairwise F_{ST} analysis and AMOVA in *Z. tau* from different fruit types

Fruit types	Genetic difference F_{ST}			Source of variation	df	Sum of squares	% variation	F -statistic
	Luffa	Bitter gourd	Winter melon					
Luffa	-			Among populations	2	3.146	8.51	$F_{ST}=0.085^*$
Bitter gourd	0.087*	-						
Winter melon	0.058	0.109*	-	Within populations	94	36.936	91.50	

* $p < 0.05$

For investigation of fruit host-related morphological differences, geometric analysis revealed the morphological differences of *Z. tau* populations among fruit types. The discriminant analysis (Figure 3) demonstrated the total discrimination by two discriminant factors for each sex (DF1 = 53%, DF2 = 47% for male; DF1 = 64%, DF2 = 36% for female). The differences in each host fruit may be associated with biological effects such as food sources and habitats, indicating the presence of adaptation (and/or a directional selection) (Clarke *et al.*, 2005). Different fruit species differ in nutritional

composition, chemistry, etc. (Abrams, 2006; Friberg and Wiklund, 2009) which can affect larval adaptation that might relate to morphological differences of adult fruit fly. In this study, three fruit species differ in genetics (Nimmakayala *et al.*, 2017) and nutrient compositions which indicate that adaptation has played an important role during larval stage and induced the morphological differences of *Z. tau* adult. The adaptation in *Z. tau* affects only morphological divergence but not genetic difference which indicating dispersal ability and no barrier to gene flow in *Z. tau* among fruit types.

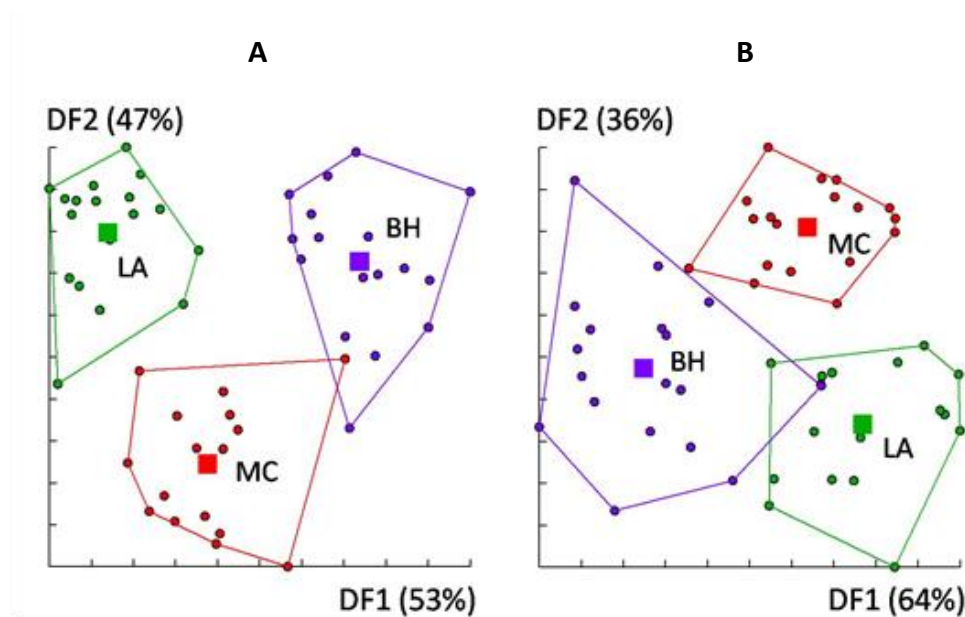


Figure 3 Scatter plot of discriminant analysis performed on *Z. tau* (a) males and (b) females. Each color represents *Z. tau* population from different fruit types: luffa (green, labeled LA), bitter gourd (red, labeled MC) and winter melon (BH).

AMOVA analysis revealed low F_{ST} value (0.085) of the genetic differences among *Z. tau* populations from different fruit types implying little barriers to gene flow and potential for dispersion among host plants. Despite of gene flow among populations, morphological differences among *Z. tau* populations were found in relation to host fruit types. These differences could be induced by their host fruit conditions during their larval developments.

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