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GENETIC VARIATION IN TWO SPECIES OF FRUIT FLIES OF THE GENUS *BACTROCERA* (DACINAE: TEPHRITIDAE: DIPTERA)

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ABSTRACT

Multilocus enzyme electrophoresis data from 14 orthologous loci (25 alleles) were used to infer the genetic variation between the two Tephritidae species, *Bactrocera (Dacus*) *cucurbitae* and *B.* (*D.*) *dorsalis*, which are common pests of vegetables and fruits in India. Out of fourteen loci analyzed, seven loci were polymorphic in *B. cucurbitae* and nine loci were polymorphic in *B. dorsalis.* The amount of observed (0.18) and expected heterozygosity (0.26) in *B. cucurbitae* were lower than the observed (0.33) and expected heterozygosity (0.40), in *B. dorsalis*. The estimates of genetic variation in terms of observed and expected heterozygosity were higher as compared to the general low level of variability found in tephritid fruit flies. Nei's genetic identity value (0.833) reveals a very close genetic similarity between the two species.

KEYWORDS:Allozymes, *Dacus,* molecular markers, heterozygosity, Hardy-Weinberg equilibrium, genetic identity

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INTRODUCTION

The family Tephritidae, the true fruit flies, is one of the most economically important dipteran families. Most of the species are pest of soft fruits, including many commercial fruits. Because of their economical importance, detailed research has been carried out on their physiology, ecology, genetics and evolution[1,2]. Fruit flies are represented in all world regions, but the most serious pest species in India are *Bactrocera dorsalis, B. cucurbitae, B. zonata* and *B. diversa*. Electrophoretic studies have proved to be a powerful tool for the analysis of genetic variability in natural populations and laboratory colonies of many tephritid flies[3-21]. In this paper we have attempted to elucidate the relationship between both the species of the same genera, *B. cucurbitae* and *B. dorsalis* using the multilocus enzyme electrophoretic approach (MLEEE), one of the most widely used molecular markers.

MATERIALS AND METHODS

Species sample

Larvae of *B. cucurbitae* (Coquillett) and *B. dorsalis* (Hendel) were collected from sponge gourd (*Luffa aegyptiaca*) and guava (*Psidium guajava*), respectively from Allahabad city (India) and wild samples were screened (and that these were reared in captivity only until emergence to confirm the correct identification of the species).

Electrophoretic procedures

Single larvae were homogenized in 0.35 ml of chilled double distilled water, homogenates were centrifuged and the supernatant was used for enzyme separation. Electrophoresis was performed on 7% polyacrylamide gel at 4^0C. 50 individuals were assayed for activity at 14 orthologous loci namely, ACPH-1, ACPH-3, ACPH-4, APH-2, EST-1, EST-3, MDH-2, ME, AO-1, AO-2, XDH-1, XDH-2, LDH. All the considered loci produced consistent interpretable banding patterns in both the species studied and the determination of isozyme locus homologies was unambiguous. Sample preparation and electrophoretic conditions for enzyme separation were according to the method of Singh et al[22]. The enzyme system studied, gel buffer and the staining solutions used are according to Ayala et al[42] and Tsukamoto[43].

Data analysis

Multiple loci were numbered in order of ascending migration distance from the origin. The relative mobility of each band was calculated and expressed as an R_f value (x100), according to the method of Tsukamoto and Horio[23].

$$R_f = \frac{\text{Migration distance of a band}}{\text{Migration distance of the (buffer) front}} \times 100$$

Gels incubated in staining solution without the corresponding substrates for all the above mentioned enzymes were used as control. Genetic interpretation has been made by conventional methods i.e. single bands indicate homozygote and multiple banded phenotypes represent heterozygote. At polymorphic loci, groups of similar R_f values were considered to represent a single allele[24]. Genotype information for each enzyme was used to calculate the frequencies of alleles in the two species. On the basis of allele frequencies, the genetic variability was estimated using the percent polymorphic loci (P), mean number of alleles per locus, mean observed (H_O) and expected (H_E) heterozygosity[25]. Chi-Square test was used to calculate the deviations in the distribution of electrophoretic phenotypes from Hardy-Weinberg equilibrium. Nei's genetic identity and genetic distance values were calculated to analyze the similarity between the two species.

RESULTS

The fourteen genetic loci analyzed, comprised of twenty five putative alleles. Enzyme activity was found at a single locus for ME and LDH in *B. cucurbitae* and for ACPH, APH, ME and LDH in *B. dorsalis*. Multiple zones of activity were observed for ACPH (ACPH-1, ACPH-2, ACPH-3, ACPH-4), APH (APH-1, APH-2, APH-3, APH-4), EST (EST-1, EST-2, EST-3, EST-4, EST-5),

AO (AO-1, AO-2), MDH (MDH-1, MDH-2, MDH-3) and XDH (XDH-1, XDH-2) loci. The enzyme activity at APH-1, APH-3, APH-4, EST-2, EST-4, EST-5, MDH-1 and MDH-3 were not consistent in all the individuals, hence does not considered for the genetic analysis due to their poor resolution. The six loci viz., ACPH-1, ACPH-2, AO-1, AO-2, XDH-1 and XDH-2 for *B. cucurbitae* and two loci viz., AO-1 and XDH-1 for *B. dorsalis* were found to be monomorphic, while the rest of the loci had showed polymorphism for three electrophoretic phenotypes.Genotype frequency at ACPH-4, EST-3 and LDH for *B. cucurbitae* and EST-1, EST-3, MDH-2, ME and LDH for *B. dorsalis* were found to be in accordance with the Hardy-Weinberg equilibrium, while others reveal significant deviations. The genotype frequencies at 50% of the polymorphic loci were found to be at Hardy-Weinberg equilibrium in the two species. The electromorph frequencies and Chi-Square values for the determination of Hardy-Weinberg equilibrium at each polymorphic locus are presented in Table-1. The genetic variability estimates in the two species (Table-2) indicates that *B. dorsalis* was found to be more polymorphic (P=81%) with a higher level of heterozygosity (H=0.33) in comparison to *B. cucurbitae*, where less variability was found (53%) with a low observed heterozygosity (H=0.18). The genetic identity (I) value derived by the method of Nei (1972) from the variation at fourteen loci was 0.833. Allozyme analysis of two species with ACPH, AO, XDH and LDH are shown in fig.1a,b,c,d,e,f,g,h respectively.

DISCUSSION

There are very few detailed studies on the gene-enzyme system in dacine fruit flies from the Oriental region. Allozyme variations have been analyzed only in *Dacus dorsalis*[26-29], *D. umbrosus*[30], *Bactrocera albistrigata*[31] and *D. cucurbitae*[32]. Out of thirteen loci studied, seven loci were polymorphic in *B. cucurbitae* and nine loci were polymorphic in *B. dorsalis*. The amount of observed (H_O) and expected (H_E) heterozygosity in *B. cucurbitae* were 0.22 and 0.34, respectively. In *B. dorsalis* the observed (H_O) and expected (H_E) heterozygosity were 0.36 and 0.44, respectively. These differences may be attributed to sampling errors. However, it is possible that these variations may be due to interspecific variations. It is interesting to note that the value of heterozygosity found in *B. cucurbitae* and *B. dorsalis* are higher than the variability found in tephritid fruit flies; H = 0.049 - 0.124[33], insects; H = 0.107[34] as well as in the invertebrates; H = 0.134[35]. There are very few instances among dipterans where, average heterozygosity is more than 20%, except for genus *Culex, Sarcophaga* and *Musca*[36,37]. However, the high values of mean heterozygosities may represent an overestimate, as Singh and Rhomberg[38] have shown that larger the number of loci under consideration, the lower is the value of heterozygosity. These values are rather high as compared to the general low level of variability found in tephritid fruit flies- H=0.049-0.138[33].The analysis of single locus heterozygosities in the two species reveals that statistically significant deviations from Hardy-Weinberg equilibrium were present at ACPH-3, APH-2, MDH-2 and ME in *B. cucurbitae* and ACPH-1, APH-2, AO-2 and XDH-2 loci in *B. dorsalis*. In all these cases there were fewer heterozygotes than expected. Such deviations have also been observed in other species of *Dacus*[26,27,29,30] and *B. albistrigata*[31]. These observations could, however, be attributed to sampling errors. The Nei's genetic identity and genetic distance values between the two species was 0.182 and 0.833 (Table 3), which indicates that they are very closely related. Such a close level of similarity is suggestive of the fact that the differentiation between the two species has been accomplished with relatively little genetic change[39,40]. Such close similarity between the two species has also been observed by Han and McPheron[41] on the analysis of nuclear ribosomal DNA.The present study shows the utility of MLEEE marker for unravelling genetic relationship in fruit flies of the genus *Bactrocera*. Further studies with allozymes and other molecular markers with these species as well as other species should be carried out in order to provide an in depth information on genetic structure of these important pests of vegetables and fruits in India.

Table 1
Electromorph frequencies and Chi-square Values in B.cucurbitae and B. dorsalis.

Locus	Electromorph	*B. cucurbitae*	*B. Dorsalis*
1. ACPH-1	a	-	0.56
(n=50)	b	-	0.44
	χ^2	-	7.18
2. ACPH-2	a	1.00	-
(n=50)	b	0.00	-
	χ^2	-	-
3. ACPH-3	a	0.59	-
(n=50)	b	0.41	-
	χ^2	12.78**	-
4. ACPH-4	a	0.50	-
(n=50)	b	0.50	-
	χ^2	4.19	-
5. APH-2	a	0.70	0.48
(n=50)	b	0.30	0.52
	χ^2	10.13**	1.02
6. EST-1	a	-	0.56
(n=50)	b	-	0.44
	χ^2	-	2.79
7. EST-3	a	0.39	0.58
(n=50)	b	0.61	0.43
	χ^2	2.02**	0.92
8. MDH-2	a	0.60	0.49
(n=50)	b	0.40	0.51
	χ^2	7.61*	1.53
9. ME	a	0.56	0.49
(n=50)	b	0.44	0.51
	χ^2	7.41*	1.53
10.AO-1	a	1.00	1.00
(n=50)	b	0.00	0.00
	χ^2	-	-
11.AO-2	a	1.00	0.59
(n=50)	b	0.00	0.41
	χ^2	-	7.74*
12.XDH-1	a	1.00	1.00
(n=50)	b	0.00	0.00
	χ^2	-	-
13.XDH-2	a	1.00	0.45
(n=50)	b	0.00	0.55
	χ^2	-	14.76**
14.LDH	a	0.48	0.51
(n=50)	b	0.52	0.49
	χ^2	1.97	0.01

$= figures in parentheses represent sample size
*χ^2= Chi Square values * p<0.05; ** p<0.01*

Table 2
Mean Heterozygosity at Allozyme Loci

Species	Mean sample size/locus	Mean no. of alleles/locus	% polymorphic loci	Mean Heterozygosity	
				Observed	Expected
B. cucurbitae	50	1.55	53.00%	0.18	0.26
B. dorsalis	50	1.81	81.00%	0.33	0.40

Table 3
Mean Genetic Identities and Genetic Distances over Allozyme Loci in B. cucurbitae and B. dorsalis

Species	*B. cucurbitae*	*B. dorsalis*
B. cucurbitae	-	0.833
B. dorsalis	0.182	-

The value below the diagonal corresponds to unbiased genetic distance and Those above the diagonal correspond to unbiased genetic identity (Nei, 1972).

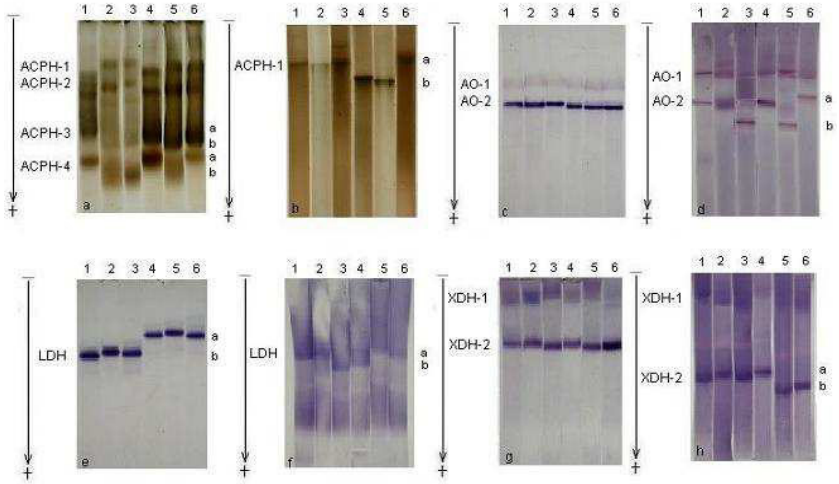

Fig. 1. Electrophoretic phenotypes of :
(a) Acid phosphatase *(B. cucurbitae)* (b) Acid phosphatase *(B. dorsalis)*
(c) Aldehyde oxidase *(B. cucurbitae)* (d) Aldehyde oxidase *(B. dorsalis)*
(e) Lactate dehydrogenase *(B. cucurbitae)* (f) Lactate dehydrogenase *(B. dorsalis)*
(g) Xanthine dehydrogenase *(B. cucurbitae)* (h) Xanthine dehydrogenase *(B. dorsalis)*

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