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ALLOZYME ANALYSIS FOR ASSESSING GENETIC VARIATION IN THE SPECIES *BACTROCERA DIVERSA AND B. ZONATA* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Allozyme analysis was used to assess genetic variability in tephritid fruit fly *Bactrocera diversa* and *B. zonata*. In our experimental conditions, allozyme analysis at twelve enzyme loci scores twenty one putative alleles. The amount of mean observed heterozygosity (H_O) was 0.22 and 0.28 and amount of mean expected heterozygosity (H_E) was 0.28 and 0.37, in *B. diversa* and *B. zonata,* respectively. The amounts of observed and expected heterozygosity were found to be higher as compared to the general low level of variability found in tephritid flies. Nei's genetic identity and distance value reveals very close similarity between the two species.

KEYWORDS

Allozyme, *Bactrocera*, Tephritidae, Heterozygosity, Molecular markers

INTRODUCTION

Fruit flies belonging to Dacinae, one of the major subfamilies of family Tephritidae, are biologically interesting and economically important group of Diptera. These comprise one of the most important global groups of pests attacking fruits and vegetable crops. This damage limits the production and successful cultivation of the affected crops[1,2]. The flies of the genus *Bactrocera (=Dacus)* are widely distributed throughout India. The most serious pest species in India are *Bactrocera (=Dacus) dorsalis, B. (=D.) zonata, B. (=D.) cucurbitae* and *B. (=D.) diversa*. These flies infest and damage ripening fruits and vegetables such as mango, peach, guava, musk melon, gourds, pumpkin, tomato etc. The damage is caused due to larvae which by feeding on almost ripe fruits make the fruits inedible and/or unmarketable. Several molecular markers e.g. allozymes, RAPD-PCR, PCR-RFLP, SSCP, SNP, microsatellites and mitochondrial DNA sequences viz., COI, COII, ND-1, ND-5 etc., have been extensively used to unravel intra and inter-specific genetic variations, population structure, migration pattern and phylogenetic relationships among various fruit fly genera, e.g. *Rhagoletis, Anastrepha, Ceratitis* and *Bactrocera (=Dacus)* of the family Tephritidae[3-16]. In the present study we used Allozymes to assess intra and interspecific genetic variation in *B. diversa* and *B. zonata*.

MATERIALS AND METHODS

Larvae of *B. diversa* (Coquillett) and *B. zonata* (Saunders) were collected from bottle gourd (*Luffa cylindrica*) and mango (*Mangifera indica*), respectively, and laboratory colonies were established and maintained at 26±1^{0}C.

ALLOZYME ANALYSIS:

For allozyme analysis eight gene-enzyme system were studied, which were as follows, ACPH-1, ACPH-3, ACPH-4 (E.C.3.1.3.2 acid phosphatase), APH-2 (E.C.3.1.3.1 alkaline phosphatase), EST-1, EST-3 (E.C.3.1.1.1 esterase), MDH-2 (E.C.1.1.1.37 malate dehydrogenase), ME (E.C.12.3.1 malic enzyme), AO-1, AO-2 (E.C.1.1.1.40 aldehyde oxidase), XDH-1, XDH-2 (E.C.1.1.1.27 xanthine dehydrogenase) and LDH (E.C.1.2.1.37 lactate dehydrogenase). Sample preparation and electrophoresis conditions for enzyme separation were conducted according to the method of Tewari and Thakur[17,18]. The staining protocols of Ayala *et al*[19] and Tsukamoto[20] were followed for the analysis of enzyme activity. The relative mobility of each band was calculated and expressed as R_f value (x100), according to the method of Tskamoto and Horio[21].

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

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[22]. Genotype information for each enzyme was used to calculate 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 [23]. Chi-Square test was used to calculate the deviations in 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 twelve genetic loci studied in two species revealed twenty one putative alleles at eight gene-enzyme system. ME and LDH show enzyme activity at a single locus while other enzymes show enzyme activity at multiple loci in both the species. The enzyme activity at ACPH and APH locus was confined to a single zone in *B. zonata*, while four zones of activity were found at ACPH and APH locus in *B. diversa*. Out of four loci found at ACPH and APH locus in *B. diversa,* two loci for ACPH (ACPH-1, ACPH-2) and one for APH (APH-2) was considered, because of its consistency in all the individuals. AO-1, AO-2, XDH-1 and XDH-2 in *B. diversa* and AO-1 and XDH-1 in *B. zonata,* were found to be monomorphic, while all the other loci had showed polymorphism for both the species.

Genotype frequency at ACPH-2, APH-2, EST-3, ME and LDH loci in *B. diversa* and APH-2, EST-1, EST-3, MDH-2, ME and LDH loci in *B. zonata* were found to be in accordance with the Hardy-Weinberg equilibrium. While, all the other loci reveal significant deviations from Hardy-Weinberg equilibrium, the genotype frequencies at 61% of the polymorphic loci were found to be at Hardy-Weinberg equilibrium in both the species. Allele frequencies and Chi-square values are presented in Table 1. The genetic variability estimates in the two species are shown in Table 2. The genetic identity (I) value derived by the method of Nei[23] from the variation at eight enzyme loci was 0.848. Allozyme analysis of two species with ACPH and ME are shown in Fig. 1a, b and 2a, b respectively.

Table 1
Allele frequencies and Chi-square values in B. diversa and B. zonata.

Locus	Allele	*B. diversa*	*B. zonata*
1. ACPH-1 (n=50)	a	0.47	0.56
	b	0.53	0.44
	X^2	9.99**	8.9*
2. ACPH-2 (n=50)	a	0.48	-
	b	0.52	-
	X^2	4.64	-
3. APH-2 (n=50)	a	0.66	0.57
	b	0.34	0.43
	X^2	1.82	1.48
4. EST-1 (n=50)	a	-	0.55
	b	-	0.45
	X^2	-	1.31
5. EST-3 (n=50)	a	0.42	0.52
	b	0.58	0.48
	X^2	3.49	0.24

6. MDH-2 (n=50)	a	0.54	0.48
	b	0.46	0.52
	χ^2	11.01**	0.32
7. ME (n=50)	a	0.46	0.49
	b	0.54	0.51
	χ^2	0.19	0.32
8. AO-1 (n=50)	a	1.00	1.00
	b	0.00	0.00
	χ^2	-	-
9. AO-2 (n=50)	a	1.00	0.56
	b	0.00	0.44
	χ^2	-	11.01**
10. XDH-1 (n=50)	a	1.00	1.00
	b	0.00	0.00
	χ^2	-	-
11. XDH-2 (n=50)	a	1.00	0.53
	b	0.00	0.47
	χ^2	-	11.60**
12. LDH (n=50)	a	0.51	0.47
	b	0.49	0.53
	χ^2	1.12	0.32

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

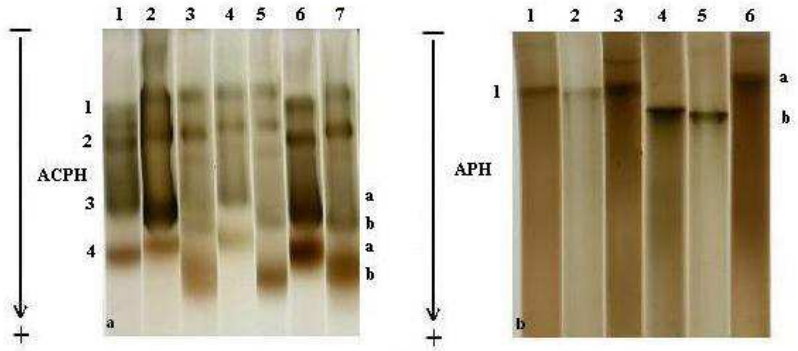

Fig. 1. Electrophoretic phenotypes of (a) Acid phosphatase (*B. diversa*)
(b) Acid phosphatase (*B. zonata*)

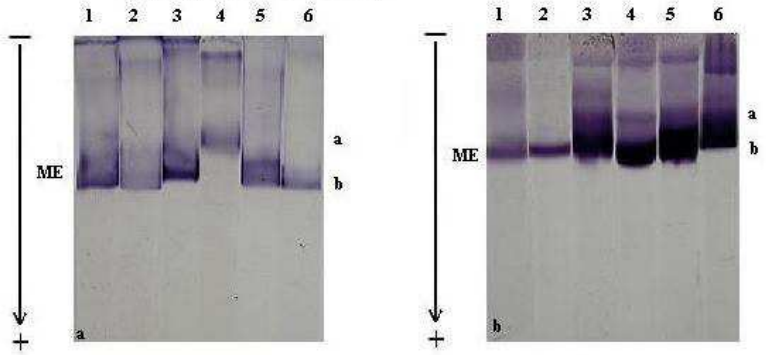

Fig. 2. Electrophoretic phenotype of (a) Malic enzyme (*B. diversa*)
(b) Malic enzyme (*B. zonata*)

DISCUSSION

Out of twelve loci studied, seven loci in *B. diversa* and nine loci in *B. zonata* were found to be polymorphic. The amount of observed (H_O) and expected (H_E) heterozygosity in *B. diversa* were 0.22 and 0.28, respectively. In *B. zonata* the observed (H_O) and expected (H_E) heterozygosity were 0.28 and 0.37, 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. diversa* and *B. zonata* are higher than the variability found in tephritid fruit flies; H = 0.049 - 0.124[24], insects; H = 0.107[25] as well as in the invertebrates; H = 0.134[26]. The analysis of single locus heterozygosity in the two species reveals that statistically significant deviations from Hardy-Weinberg equilibrium were present at ACPH-1 and MDH-2 in *B. diversa* and ACPH-1, AO-2 and XDH-2 loci in *B. zonata*. In all these cases there were fewer heterozygotes than expected. Such deviations have also been observed in other species of *Dacus* and *B. albistrigata*[27,28,29,30]. These observations could, however, be attributed to sampling errors.

The genetic identity (I) valued derived by the method of Nei[23] from the variation at eight enzyme loci between the two species was 0.848 indicate that they are very closely related (Table 3). 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[18,31,32].

CONCLUSION

In the present study allozymes are proved to be powerful technique to discriminate two *Bactrocera* species. Also, it will provide support to taxonomist, as it reveals information based on genetic constitution. Further studies with allozymes and other molecular markers should be carried out to unravel genetic variation and genetic relatedness among these economically important flies.

Table – 2
Mean Heterozygosity at Allozyme Loci.

Species	Mean sample size/locus	Mean no. of alleles/locus	% polymorphic	Mean heterozygosity	
loci				Observed	Expected
B. diversa	50	2.00	58.00%	0.22	0.28
B. zonata	50	2.00	75.00%	0.28	0.37

Table – 3
Mean Genetic Identities and Genetic Distances over Allozyme Loci in B. diversa and B. zonata.

Species	*Bactrocera diversa*	*Bactrocera zonata*
B. diversa	**	0.848
B. zonata	0.163	**

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

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