# PHYLOGENETIC RELATIONSHIP OF AN INVASIVE DROSOPHILID, *Zaprionus indianus* AND CLOSELY RELATED SPECIES OF DROSOPHILIDAE (DIPTERA) BASED ON ESTERASE PATTERNS

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*Zaprionus indianus* (Gupta, 1970) is a drosophilid belongs to the genus and subgenus *Zaprionus* (*Drosophilidae*). It is a new potential pest for numerous fruit crops that exhibits a wide geographical distribution throughout the tropics and temperate regions. Based on the various locations where this organism has been found, it is believed that *Z. indianus* lives on 80 host plants, making this species the most ecologically diverse drosophilid (Yassin and David, 2010).

Drosophilids are saprophagic species that develop in decomposing plant material including fruits, leaves and flowers as well as fungi. Species of the group *melanogaster* tend to use decomposing fruits, flowers and other plant parts as substrates for feeding and mating. Species of the genus *Zaprionus* also mate on flowers and fruits (Schmitz *et al.*, 2007; Markow and O'Grady, 2006 & 2008). In addition, *Z. indianus* feeds on the bacteria and yeast found in decomposing fruits, principally on the yeast *Candida tropicalis* (Gomes *et al.*, 2003). There have been proposals to reclassify the genus *Zaprionus* as a subgenus or group of the genus *Drosophila* because various molecular markers have indicated a close relationship between *Zaprionus* species and the immigrans-*Hirtodrosophila* radiation within *Drosophila*. These markers, together with alloenzymes and quantitative traits, have been used to describe the probable scenario for the expansion of *Z. indianus* from its center of dispersal (Africa) to regions of Asia (ancient dispersal) and the Americas (recent dispersal) (Commar *et al*., 2012). Due to its evolutionary history, ecological and morphological diversity, the *Zaprionus* genus seems to be a good model for comparative studies with the *melanogaster* subgroup. The similarities between species of the genus *Zaprionus* and species of the subgroup *melanogaster* in terms of their evolutionary characteristics and their ecological diversity have been highlighted in evolutionary studies (De Setta *et al.*, 2009; 2011). Although the phylogenetic relationships within the *Zaprionus* genus had been recently proposed (Yassin *et al.*, 2008), its taxonomic positioning in the *Drosophilidae* family remains a matter for discussion.

Isozyme patterns showed a pronounced differentiation in many organ-

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isms including insects. They are still amongst the quickest and cheapest marker systems to develop, and remain an excellent choice to identify low levels of genetic variation (Ferguson *et al.*, 1995). Esterase isozyme is one of the lipid-hydrolyzing enzymes which have a great significance in the field of genetics and toxicology (Callaghan *et al.*, 1994). In insects, esterase genes have shown high rates of intraspecific and interspecific variation. The level of insect esterase may found to be highly variable depending on the life stage, sex, tissue, hormones, strain, food, environmental conditions and numerous other factors (Devorshak and Roe, 1999; Villatte and Bachmann, 2002; Li *et al.*, 2005; Baffi *et al.*, 2007). All these studies have suggested that the esterase isozymes exhibit high level of polymorphism in *Drosophila* and other organisms, and this polymorphism offers adaptive flexibilities to these species.

This study aimed to focus on the phylogenetic relationship among *D. melanogaster*, its sibling species *D. simulans* and *Z. indianus*, a divergent species belonging to *Drosophilidae*. Two natural populations of *Z. indianus* and natural populations of *D. melanogaster* and *D. simulans*; belonging to the *melanogaster* species group, were used. A polyacrylamide gel electrophoresis was applied to study the esterase isozyme banding patterns in the four populations compared to *D. melanogaster* (Oregon-K strain) as a standard laboratory strain.

## MATERIALS AND METHODS

### *Insects*

Two natural populations of *Z. indianus* as well as wild type flies of *D. melanogaster* and *D. simulans* were collected from Kafr El-Sheikh Governorate, Egypt. Samples of *Z. indianus* adults were collected from two different cities in Kafr El-Sheikh localities; KafrEl-Sheikh (A) and Sidi Salem (B). A stock colony was established from the natural collected flies and maintained at 25±2°C on the standard *Drosophila* medium (cornmeal, agar, molasses, yeast and anti-fungal agent; propionic acid). *Drosophila melanogaster* (Oregon-K) stock population was used as a standard laboratory strain for comparison.

### *Polyacrylamide gel electrophoresis (PAGE)*

Electrophoretic patterns of esterase isozymes were studied for both wild type natural populations and standard laboratory strain. Samples were prepared from whole body of adults by homogenizing 250 mg flies in 500 µl of 20% sucrose according to El-Fadly *et al.* (1990). Homogenates were centrifuged at 12000 rpm for 15 min at 4°C.

Esterase isozyme patterns were analyzed using 7.5% polyacrylamide gel electrophoresis according to the method of Davis (1964). An equal volume (30 µl) of supernatant was carefully loaded to each well. Esterase bands were detected on the gel as described by Vallejos (1983) using α-naphthyl acetate as substrate and subse-

quent color development with fast blue RR salts. After the appearance of bands, the gels were photographed.

***Phylogenetic analysis***

The data presented in Table (1) generated from esterase isozyme banding patterns were introduced to SPSS package program according to binary values of (1) and (0) for the presence and absence of bands, respectively. The genetic distances among the genotypes were assessed based on Jaccard's similarity coefficient (Jaccard, 1901) using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) analysis (Nei, 1973).

## RESULTS AND DISCUSSION

***Esterase polymorphism***

The electrophoresis results of esterase isozyme patterns presented in Fig. (1) give an evidence of esterase polymorphisms of the three analyzed species under investigation. Figure (1) shows that natural populations of *D. melanogaster* and *D. simulans* (from the *melanogaster* subgroup), *Z. indianus* (from *Zaprionus* genus) and the standard *D. melanogaster* laboratory strain (Oregon-K) show a high degree of polymorphism. A total of 18 bands were detected (Table 1), two bands were monomorphic and the other 16 bands were polymorphic, with 88.89% polymorphism. As it appears in Fig. (1), *D. melanogaster* (Oregon-K) is having seven polymorphic bands, *D. melanogaster* and *D. simulans* are having each four polymorphic bands, and at last *Z. indianus* populations; A and B are having eight and ten polymorphic bands, respectively.

On the other hand, the electrophoretic bands showed wide variation in their intensities ranging from faint to dark, reflecting different activities in the tissues of these populations.

The observed isozyme patterns can be explained in terms of allelic differences. Isozymes are all functionally similar forms of enzyme including all polymers of subunits produced by different gene loci or by different alleles at the same locus. Their electrophoretic mobilities are the result of different size and shapes of enzyme molecules and their variation is a good indicator of genetic diversity (Shannon, 1968). So, electrophoresis separation of isozymes has been widely used both in taxonomic and genetic studies in *Drosophila.* In this regard, Galego *et al.* (2006) described six loci coding for esterases in *Z. indianus*, four of which encode α-esterases and two encode β-esterases. Two of these loci, Est-3 (four alleles) and Est-2 (two alleles), were polymorphic. Alloenzyme studies indicate that the distribution of genetic variability at the α-esterase 3 locus in *Z. indianus* is influenced by natural selection, including selection by insecticides and selection stemming from climatic variation (Galego and Carareto, 2007 & 2010). Plasticity in the distribution of allele frequencies for the Est-3 locus may also have contributed to the successful spread of this organism given that esterases perform multiple essential functions in insects.

***Phylogenetic relationship***

It has been noticed that the number of species specific bands ranged from 6 bands (for *D. melanogaster* and *D. simulans*) to 12 bands (for *Z. indianus*; population B). Each esterase band was considered as a separate character and scored 1 (present) or 0 (absent) to obtain a rectangular binary data matrix (Table 1).

A similarity matrix of the species studied was obtained using Jaccard's coefficient. As it appears from the data in Table (2), the maximum similarity coefficient of 0.692 was found between the two *Z. indianus* populations; A and B, indicating a high degree of genetic similarity between them.

The relatedness between the species studied for this investigation was calculated by Jaccard's similarity coefficient method using the above data mentioned in Table (2). Phylogenetic analysis based on esterase isozymes profile was constructed using the UPGMA procedure (Fig. 2).

Figure (2) shows that *D. melanogaster* (the laboratory strain; Oregon-K and the natural population) and *D. simulans* form one cluster unit of the melanogaster species group whereas the other cluster unit consists of the two *Z. indianus* populations; A and B. The phylogenetic tree reveals that there is a high degree of diversity existing between *Zaprionus* and *melanogaster* species.

These results support the previous taxonomical data obtained from morphological and cyto-taxonomical analysis that *D. simulans* show close similarity with *D. melanogaster* (Capy and Gibert, 2004; Nolte and Schlötterer, 2008). This is expected since these species are closely related. Rakshit and Chatterjee (2012) also noted that the evolutionary distance of *Z. indianus* is far away from melanogaster species group of *Drosophila*.

Esterase patterns are important tool for genetic differentiation analysis and evolutionary relationship of *Drosophila* species (Nascimento and De Campos Bicudo, 2002). Commar *et al.* (2012) mentioned that various molecular markers with alloenzymes and quantitative traits have indicated a close relationship between *Zaprionus* species and the *immigrans-Hirtodrosophila* radiation within *Drosophila*.

Species of the subgenus *Zaprionus* and subgroup *melanogaster* would share a last common ancestor at least as old as the divergence of the *Sophophora* and *Drosophila* subgenera (Russo *et al.*, 1995; Tamura *et al.*, 2004). In addition to their shared ecological characteristics, the historic and contemporary geographic coexistence between species of the subgroup *melanogaster* and the subgenus *Zaprionus* suggest that these two groups of species passed through a period that allowed the transfer of transposable elements during their diversification. The invasive potential of various species of both genus, such as *D. melanogaster* (David and Capy, 1988), *D. simulans* (Hamblin and Veuille, 1999) and *Z. indianus* (Gupta, 1970) may

have promoted horizontal transfer events (Carareto, 2011).

*Drosophila melanogaster* and *Drosophila simulans*as were considered the dominant species before the invasion of *Z. indianus* (Valente *et al.,* 1989; Santos and Valente, 1990; Valiati and Valente, 1996).

## SUMMARY

Two natural populations of *Zaprionus indianus*; collected from two different cities in Kafr El-Sheikh governorate, Egypt, were analyzed for esterase variability in comparison with natural population of both of *D. melanogaster* and *D. simulans*. *Drosophila melanogaster* (Oregon-K) was also used as a standard laboratory strain. The electrophoresis results gave an evidence of esterase polymorphisms in the studied populations with 88.89% polymorphism. Phylogenetic analysis based on Jaccard's similarity coefficient of esterase patterns showed that *D. melanogaster* and *D. simulans* populations formed one cluster unit of the *melanogaster* species group whereas other cluster unit consisted of the two *Z. indianus* populations.

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Table (1): Presence and absence of esterase bands of studied *Drosophilidae* species.

| | *Drosophila melanogaster* (Oregon-K) | *Drosophila melanogaster* | *Drosophila simulans* | *Zaprionus indianus* (A) | *Zaprionus indianus* (B) |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | 0 | 0 | 0 | 1 | 1 |
| 3 | 0 | 0 | 0 | 0 | 1 |
| 4 | 0 | 0 | 0 | 1 | 1 |
| 5 | 1 | 0 | 0 | 1 | 1 |
| 6 | 1 | 1 | 0 | 1 | 0 |
| 7 | 0 | 0 | 1 | 1 | 1 |
| 8 | 1 | 1 | 1 | 0 | 0 |
| 9 | 1 | 0 | 0 | 1 | 1 |
| 10 | 1 | 0 | 0 | 0 | 1 |
| 11 | 0 | 0 | 1 | 0 | 0 |
| 12 | 0 | 1 | 0 | 1 | 1 |
| 13 | 1 | 0 | 1 | 0 | 0 |
| 14 | 0 | 1 | 0 | 0 | 0 |
| 15 | 1 | 1 | 1 | 1 | 1 |
| 16 | 0 | 0 | 0 | 0 | 1 |
| 17 | 1 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 1 | 1 |
| Total | 9 | 6 | 6 | 10 | 12 |

Table (2): Jaccard similarity coefficient of studied Drosophilidae species calculated from esterase banding patterns.

| | *Drosophila melanogaster* (Oregon-K) | *Drosophila melanogaster* | *Drosophila simulans* | *Zaprionus indianus* (A) |
|---|---|---|---|---|
| *D. melanogaster* | 0.364 | | | |
| *D. simulans* | 0.364 | 0.333 | | |
| *Z. indianus* (A) | 0.357 | 0.333 | 0.231 | |
| *Z. indianus* (B) | 0.312 | 0.200 | 0.200 | 0.692 |

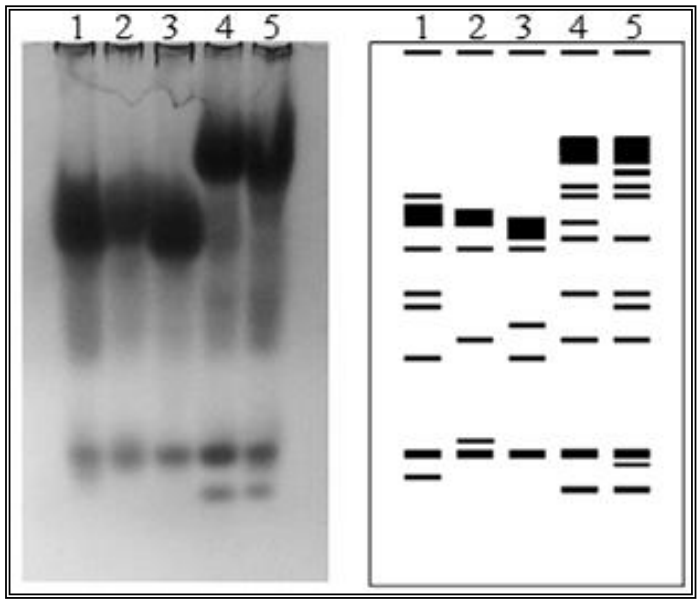


Fig. (1): Isozymes profile of α-esterase of different *Drosophilidae* species. Lane 1: *D. melanogaster* (Oregon-K); Lane 2: *D. melanogaster*; Lane 3: *D. simulans*; Lane 4: *Z. indianus* (A); Lane 5: *Z. indianus* (B).

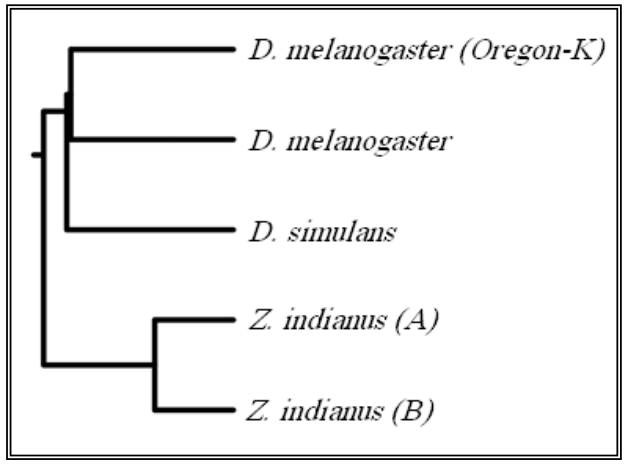


Fig. (2): UPGMA phylogenetic tree resembles the phylogenetic relationship between the different *Drosophilidae* species based on Jaccard similarity indices.