Review Article

Taxonomic and evolutionary analysis of *Zaprionus indianus* and its colonization of Palearctic and Neotropical regions

Leliane Silva Commar[1], Luis Gustavo da Conceição Galego[2], Carlos Roberto Ceron[3] and Claudia Marcia Aparecida Carareto[1]

[1]*Departamento de Biologia, Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto, SP, Brazil.*
[2]*Departamento de Ciências Biológicas, Instituto de Ciências Exatas e Naturais, Universidade Federal do Triângulo Mineiro, Uberlândia, MG, Brazil.*
[3]*Departamento de Química e Ciências Ambientais, Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto, SP, Brazil.*

Abstract

Zaprionus indianus is a dipteran (Drosophilidae) with a wide distribution throughout the tropics and temperate Palearctic and Nearctic regions. 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 *Zaprionus indianus* from its center of dispersal (Africa) to regions of Asia (ancient dispersal) and the Americas (recent dispersal). The introduction of *Z. indianus* into Brazil was first reported in 1999 and the current consensus is that the introduced flies came from high-latitude African populations through the importation of fruit. Once in Brazil, *Z. indianus* spread rapidly throughout the Southeast and then to the rest of the country, in association with highway-based fruit commerce. These and other aspects of the evolutionary biology of *Z. indianus* are addressed in this review, including a description of a probable route for this species' dispersal during its recent expansion.

Key words: alloenzyme, bioinvasion, molecular markers, phylogenetic analysis, quantitative traits.

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Introduction

A little more than 10 years ago, *Zaprionus indianus* (Gupta, 1970)*,* a drosophilid belonging to the genus *Zaprionus* and the subgenus of the same name, was introduced into Brazil and became a major pest affecting fig production (Vilela, 1999) giving rise to its Brazilian common name of fig fly. Vilela *et al.* (2001) and Stein *et al.* (2003) provided detailed descriptions of the species immediately after its introduction*.* Briefly, *Z. indianus* is approximately 3 mm long, has red eyes and a brown body with longitudinal white bands interspersed with black bands on the back of the head and thorax. In addition to being a human commensal, *Z. indianus* is a generalist species that uses a variety of endemic and introduced fruits as sites for mating and oviposition (Lachaise and Tsacas, 1983; Schmitz *et al.*, 2007). *Zaprionus indianus* feeds on the bacteria and yeast found in decomposing fruits, principally on the yeast *Candida tropicalis* (Gomes *et al.*, 2003). Based on the vari-

ous 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 in the Afrotropical fauna (Yassin and David, 2010).This generalist characteristic is perhaps one of the principal factors contributing to the success of *Z. indianus* in tropical and subtropical regions.

There has been much speculation about the phylogenetic position of *Z. indianus* within the genus and subgenus *Zaprionus* (Drosophilidae). This species has aroused great interest in the Brazilian scientific community because of its recent introduction and rapid dispersal, first throughout Brazil and then across a large part of the South American continent. These and other aspects of the evolutionary biology of *Z. indianus* are addressed in this review, which includes an attempt to trace a probable route of dispersal for this species during its recent expansion.

Phylogenetic relationships of the genus *Zaprionus*

The genus *Zaprionus* is divided into two subgenera that are distinguished by their geographic origin: the subge-

Send correspondence to Claudia M.A. Carareto. Departamento de Biologia, Universidade Estadual Paulista (UNESP), São José Rio Preto, 15054-000, SP, Brazil. E-mail: carareto@ibilce.unesp.br.

nus *Anaprionus* (Okada, 1990) contains 10 species from the Oriental biogeographic region (Okada and Carson, 1983; Wynn and Toda, 1988; Gupta and Gupta, 1991) and the subgenus *Zaprionus* comprises 49 essentially Afrotropical species (Okada and Carson, 1983; Yassin *et al.*, 2008a,b). Chassagnard and Tsacas (1993) classified the species of the subgenus *Zaprionus* into two groups, *inermis* and *armatus*, with the latter comprising three subgroups: *armatus*, *tuberculatus* and *vittiger*. Recent phylogenetic revisions using molecular and morphological characters have shown *Zaprionus* s.s. species to be monophyletic, but both species groups to be polyphyletic (Yassin *et al.*, 2008a). Based on these recent phylogenetic findings, a new classification of the subgenus *Zaprionus* has been proposed and includes a redefinition of the boundaries of the *armatus* and *inermis* species groups. The *vittiger* subgroup was upgraded to the level of a species group and the *tuberculatus* subgroup was transferred from the *armatus* to the *inermis* group (Yassin and David, 2010). *Zaprionus indianus* was included in the *armatus* group and *vittiger* subgroup (now group) by Chassagnard (1996), as mentioned above. Gupta (1970) proposed the epithet *indianus* for the species, probably because the type specimen used for identification came from India; he was probably unaware of the distribution of the species throughout the entire Afrotropical region (Vilela *et al.*, 2001). This was not, however, the only misunderstanding related to the identification of this species. Tsacas (1985) reviewed all of the problems concerning the nomenclature of *Z. indianus* and pointed out that synonymous species names include *Z. inermis* (Séguy, 1983), *Z. paravittiger* (Goodbole and Vaidya, 1972) and *Z. collarti* (Tsacas, 1980). He also noted that *Z. vittiger* (Coquillet, 1901) can easily be misidentified as *Z. indianus*.

The genus *Zaprionus* has also been the subject of much discussion regarding its proper phylogenetic position within the Drosophilidae. The first attempt to establish phylogenetic relationships within this family was by Throckmorton (1962, 1975). Using biogeographic, anatomical and behavioral data, Throckmorton (1975) characterized the Drosophilidae as a paraphyletic group and considered *Zaprionus* to be a subgenus of *Drosophila* within the *immigrans-Hirtodrosophila* radiation. Throckmorton's classification was criticized because he did not use the concept of monophyly. Other researchers proposed new phylogenetic relationships among drosophilids. For example, Grimaldi (1990) used cladistic analysis to construct a phylogenetic tree for *Drosophila* and related genera based on 217 morphological characteristics of 120 representative species. In this phylogeny, the subgenera *Hirtodrosophila, Scaptomyza, Idyomia* and *Zaprionus* were excluded from the genus *Drosophila*. Despite the fact that mitochondrial DNA analyses by De Salle (1992) corroborated Grimaldi's proposal, the majority of phylogenies that were constructed thereafter based on molecular markers conflicted with these

authors proposals and frequently placed species of *Zaprionus* within the genus *Drosophila*.

Three of the early molecular phylogenies included species from *Zaprionus* on a branch between the subgenera *Drosophila* and *Sophophora* of the genus *Drosophila*. These phylogenies were constructed using the gene sequences of the alcohol dehydrogenase enzyme (*Adh*) (Thomas and Hunt, 1993), copper/zinc superoxide dismutase *(*Kwiatowski *et al.*, 1994) or the two concatenated gene sequences (*Cu/Zn Sod* and *Adh*; Russo *et al.*, 1995). The great majority of phylogenetic analyses, however, link species of the genus *Zaprionus* to the subgenus *Drosophila*. Based on ribosomal RNA sequences from 72 species of Drosophilidae, Pelandakis and Solignac (1993) placed the species of *Zaprionus* (*Z. inermis, Z. sepsoides, Z. capensis, Z. taronus* and *Z. lineosus*) in a single clade within the subgenus *Drosophila,* in close proximity to the *immigrans* and *repleta* groups. Kwiatowski and Ayala (1999) subsequently used sequences of the genes *Adh, Sod* and *Gpdh* to place *Zaprionus* in the same clade as *D. immigrans.* Other analyses produced similar results, placing *Zaprionus* close to *D. immigrans* but not within the same clade. This is the case for the study by Powell and De Salle (1995), who analyzed mitochondrial and ribosomal sequences as well as morphological and behavioral data. It is also true for the study by Remsen and De Salle (1998) who, in addition to the data used by Powell and De Salle (1995), analyzed nucleotide sequences of the genes *Adh* and *Sod.* In contrast, the phylogenetic analysis by Tatarenkov *et al.* (1999), which used four nuclear markers (*Ddc, Sod, Adh* and *Gpdh*), placed *Zaprionus* (as well as *Scaptomyza*) in a position that formed a sister clade with the *virilis* and *repleta* groups. In this study, Tatarenkov *et al.* (1999) proposed for the first time that the taxon *Zaprionus* should be considered a subgenus of the genus *Drosophila*.

More recent studies, such as those of Robe *et al.* (2005), which analyzed the nuclear genes alpha methyl dopa (*amd*) and mitochondrial cytochrome oxidase II (COII), and Da Lage *et al.* (2007), which used sequences of the gene *Amyrel,* placed *Zaprionus* within the *immigrans-Hirtodrosophila* radiation of the subgenus *Drosophila,* thus corroborating Throckmorton's original proposal of 1975. At the same time, a study using the *Amyrel* gene*,* the *COII* gene and morphological characteristics has linked *Zaprionus* to the *tumiditarsus* group*,* which is basically classified as part of the subgenus *Drosophila* (Yassin, 2007).

Doubts about the phylogenetic relationships of drosophilids are not restricted to the genus *Zaprionus*. A phylogenetic reconstruction by van der Linde and Houle (2008) combined 117 trees to produce a *"supertree"* that corroborated the paraphyly of the genus *Drosophila* and placed *Zaprionus* among species of the *immigrans-tripunctata* radiation, which is currently the most accepted position. These results support a new classification for the

genus *Zaprionus* in which it is a subgenus of the genus *Drosophila* or even a group within the subgenus *Drosophila.* However, to understand the evolutionary relationships of the genus *Zaprionus* better it is necessary to perform complementary analyses that use a larger number of molecular markers and morphological characteristics. Nevertheless, most studies indicate that the diversification of *Zaprionus* occurred after the origin of the subgenus *Sophophora,* making *Zaprionus* more related to the subgenus *Drosophila* than to *Sophophora.* However, the exact phylogenetic relationship between *Zaprionus* (and other drosophilids) and the genus *Drosophila* is still a matter for speculation.

Evolution and geographic distribution of the genus *Zaprionus*

The genus *Zaprionus* is currently believed to have arisen in the Oriental region (Okada, 1981) relatively recently, during the Late Miocene (~10 million years ago), as compared to the origin of the subgenus *Drosophila* (~ 60 MYA) (Yassin *et al.*, 2008a). Using mitochondrial (*COII*) and nuclear (*Amyrel*) markers and a reconstruction of historical biogeography, Yassin *et al.* (2008a) proposed that immediately after its origin in the East, during the Quaternary (~7 MYA), an ancestral lineage of the subgenus *Zaprionus* colonized Africa via a maritime route from the islands of the Indian Ocean. Thereafter, most of the morphological and ecological diversification of the subgenus took place in West Africa during the cyclic climatic changes of the Quaternary. In this analysis, the authors adopted the Chassagnard and Tsacas (1993) classification of the subgenus *Zaprionus*. However, as commented above, the groups and subgroups have been redefined in the light of a more recent phylogenetic analysis (Yassin and David, 2010). In order to provide an overview of the diversification of the subgenus in tropical Africa, in this discussion we have followed strictly the description by Yassin *et al.* (2008a), although it is important to note that the *tuberculatus* subgroup was transferred from the *armatus* to the *inermis* group and the *vittiger* subgroup (from the *armatus* group) was upgraded to species level. In short, the two groups of the subgenus *Zaprionus* evolved in tropical Africa: the *inermis* group, which evolved first in the islands of the Indian Ocean (6.9 ± 0.8 MYA) but with many independent dispersal events between the African continent and these islands, especially during the Pleistocene, and the *armatus* group, which appeared later (4.4 ± 0.9 MYA) in Central Africa, during the Early Pliocene. This diversification of the subgenus *Zaprionus* in Africa and the islands of the Indian Ocean occurred in parallel with the evolution of the species of the subgroup *melanogaster* of the group *melanogaster* in the genus *Drosophila* (Lachaise *et al.*, 1988; Lachaise and Silvain, 2004). Recently, three distantly related Afrotropical species (*Z. indianus, Z. tuberculatus* and *Z. ghesquierei*) became invasive and have

been found in the Palearctic region (Chassagnard and Kraaijeveld, 1991). *Zaprionus indianus* is the most widespread species of the genus and occurs over a broad range on four continents (Asia, Africa and the Americas).

Ecological, ethological and evolutionary features shared between *Zaprionus* and *Drosophila* and genomic invasion by transposable elements

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). As mentioned above, the origin of the subgenus *Zaprionus* dates back to the Late Miocene (~7 MYA) in tropical Africa (Yassin *et al.*, 2008a), and the species included in this subgroup originated between 4.3 and 6.9 MYA. Interestingly, the subgroup *melanogaster* arose at the same time and in the same geographic region. The subgroup *melanogaster* diversified in tropical Africa from a proto-*melanogaster* lineage that migrated from the East about 17-20 MYA. In West Africa, the complexes *erecta* and *yakuba* evolved approximately 13-15 and 8-15 MYA, respectively, and the complex *melanogaster*, the origin of the lineages that gave rise to *D. melanogaster* on one side and to the subcomplex *simulans* on the other, emerged about 2-3 MYA. This subcomplex produced *D. simulans, D. sechellia* and *D. mauritiana*, apparently from the same diversification event, only about 400,000 years ago (Lachaise *et al.*, 1988; Lachaise and Silvain, 2004). This superposition of time and place of origin and diversification allows for evolutionary studies involving the comparison of genetic, morphological and behavioral data. Some of the studies done have involved the analysis of sequences of transposable elements (*e.g.*, *mariner, Hosimary, gypsy, copia* and *micropia*) in species of the two groups (Maruyama and Hartl, 1991; Lawrence and Hart1, 1992; Brunet *et al.*, 1994, 1999; Jordan and McDonald, 1998; Heredia *et al.*, 2004; de Almeida and Carareto, 2006; Ludwig and Loreto, 2007; Ludwig *et al.*, 2008; Vidal *et al.*, 2009; de Setta *et al.*, 2009, 2011; Deprá *et al.*, 2010). These studies found similarities between the sequences of transposable elements from the subgenus *Zaprionus* and from certain species of the subgroup *melanogaster* that were greater than the similarities between species of the same species group. In addition, these elements do not occur in other species of the group *melanogaster.* The studies cited above indicate that these elements were involved in instances of horizontal transfer between the species of the two genera.

The sharing of transposable elements via horizontal transfer requires spatial, temporal and ecological overlap. 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 (Markow and O'Grady, 2006, 2008) and feed on this material and on microorganisms involved in decomposition. These microorganisms are eliminated in the feces and deposited at mating sites and on the surfaces of eggs (Bakula, 1969; Gilbert, 1980). This environment is thus rich in potential vectors for the horizontal transfer of transposable elements such as symbiotic bacteria (Hotopp *et al.*, 2007), viruses (Fraser *et al.*, 1996) and parasites such as ticks (Gilbert, 2010), mites (Houck *et al.*, 1991) and wasps (Yoshiyama *et al.*, 2001).

In addition to their shared ecological characteristics, the historic and contemporary geographic coexistence between species of the subgroup *melanogaster* and the subgenus *Zaprionus* suggests 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 groups, such as *D. melanogaster* (David and Capy, 1988), *D. simulans* (Hamblin and Veuille, 1999), *D. malerkotliana* (Vogl *et al.*, 2003), *D. ananassae* (Val and Sene, 1980) and *Zaprionus indianus* (Gupta, 1970) may have promoted horizontal transfer events (for a detailed review, see Carareto, 2011).

Intercontinental colonization by *Zaprionus indianus*

Zaprionus indianus probably originated in Africa (Tsacas *et al.*, 1981, 1985; David *et al.*, 2006a,b; Yassin *et al.*, 2008a,b) and can be considered one of the most successful colonizing species of its genus. The rapid geographic expansion of this species has led to many hypotheses on the processes involved in this invasion. Yassin *et al.* (2008b) studied the distribution of mitochondrial haplotypes of the *COI* and *COII* genes in 23 geographically distinct populations of *Z. indianus* and detected two phylogenetic lineages. Lineage 1 included three African populations, which supported the African origin of this species. A distinct phylogenetic pattern was observed in lineage II. The Atlantic populations (of the Americas and the island of Madeira) were closer to the ancestral African populations than to those of the East (Madagascar, Middle East and India), indicating that *Z. indianus* underwent two independent radiations: an older radiation in which it spread from East Africa to the East, and a more recent radiation in which it spread to the West (via the Atlantic). The various hypotheses explaining the two great invasions (Old World, Asia; New World, Americas) are described below.

The colonization of Asia

The colonization of Asia may have occurred only 30 years ago (David *et al.*, 2006a), based on the description by Gupta (1970) using type specimens from India, or it may have occurred centuries ago (Karan *et al.*, 2000). This process is not well documented and some authors even claim that *Z. indianus* is endemic to India (Gupta, 1970) and Paki-

stan (Shakoori and Butt, 1979). However, *Z. indianus* has not been recorded in nearby Sri Lanka (Karan *et al.*, 2000). *Zaprionus indianus* has been found in the Comoro Islands, the Canary Islands and Madagascar (Chassagnard and Tsacas, 1993), as well as in Saudi Arabia (Amoudi *et al.*, 1991; 1993a,b) and in other parts of the Palearctic region (Chassagnard and Kraaijeveld, 1991).

The few records of *Z. indianus* in Asia include studies of quantitative traits and alloenzyme polymorphisms. In Indian populations, the sizes of the body, thorax and wings are reduced at higher temperatures (Karan *et al.*, 1999), and various quantitative (weight, body and wing size) and reproductive (number of ovarioles) traits increase with latitude. The quantitative traits (wing, thorax and body size) also increase with altitude (Karan *et al.*, 2000). The geographical characteristics (latitude and longitude) are not selective factors themselves, but they may be related to some form of climatic selection (Karan *et al.*, 2000). Thus, these data provide indirect evidence of the action of natural selection, probably driven by variation in temperature. Various authors have suggested that increased body size may improve flying ability (Stalker, 1980; David *et al.*, 1994; Azevedo *et al.*, 1998).

Clines related to alloenzyme polymorphisms are generally attributed to greater or lesser stability of the variants, depending on the temperature (Hedrick, 1983; Parkash and Sharma, 1993; Parkash and Yadav, 1993a). Thermoresistant variants would be at advantage in environments with a higher ambient temperature (low latitudes and altitudes), and thermosusceptible variants would be at advantage in environments with lower temperatures (higher latitudes and altitudes). Environments with significant temperature variation during the year could support populations with variants of both types or with greater than expected heterozygosity resulting from balanced selection (Parkash and Sharma, 1993; Parkash and Yadav, 1993a).

Studies of different alloenzyme markers in Indian populations of *Z. indianus* have found that the markers show latitudinal clinal variation, including, for example, polymorphisms of the alloenzymes ACPH, esterases and MDH (Parkash and Sharma,1993), Acph-1^S, Acph-1^F, Mdh-1^F, Ao^S, Adh^F, Est-1 and 2 and α-Gpdh^F (Parkash and Yadav, 1993b;Yadav and Parkash, 1993a; Parkash *et al.*, 1994) and the allele Adh^F (Yadav and Parkash, 1993b). Additionally, Parkash *et al.* (1992) found a significant increase in the frequency of the Adh^F allele with increasing latitude while Yadav and Parkash (1993b) found that this variant increases tolerance to higher ethanol concentrations.

The clinal variation found in some Asian populations of *Z. indianus* is indicative of older colonization. The details of this colonization have been completely lost. However, the association of this drosophilid with altered environments and evidence that the recent colonization of the Americas probably occurred through the intercontinental transport of fruits (David *et al.*, 2006b; Yassin *et al.*,

2009a; Galego and Carareto, 2010a), it is likely that the colonization of Asia occurred in a similar way during the great navigations at the end of the Middle Ages or beginning of the Modern period (15th century) that involved the trading of spices and other products between the East and West. There are still questions, however, regarding the dispersal of *Z. indianus* in the Palearctic regions through the international fruit trade. For example, while in the Americas the dispersion of this species was extremely rapid (in approximately six years from São Paulo to Florida), it took more than 40 years for the species to spread from India to Egypt. The population in Egypt seems to be very recent in origin, more recent than December 2002 (Yassin *et al.*, 2009a). This population may have come from a natural expansion from tropical Africa, through the Nile valley, or perhaps through trade in fruits from East Africa or Asia. Analyses of the polymorphisms of chromosomal inversions have shown that the populations in Alexandria (Egypt) are more closely related to Indian populations (Gupta and Kumar, 1987) than to African or Brazilian ones (Ananina *et al.*, 2007). In addition, quantitative analyses of alloenzymes and RAPD have revealed low genetic variability in the Egyptian populations, a characteristic of recently introduced populations (Yassin *et al.*, 2009b).

The colonization of the Americas

Vilela (1999) was the first to report the presence of *Z. indianus* in South America; the species was found in persimmons (*Diospyros kaki,* Ebenaceae) from Santa Isabel in São Paulo city in the state of São Paulo, Brazil. At the same time, the species was also collected at other locations in this state (Ribeirão Preto, São José do Rio Preto, and Valinhos) and in the Federal District (Vilela *et al.*, 2001). Two hypotheses for the introduction of this species into Brazil were initially proposed by these authors. The less likely hypothesis was that some specimens had escaped from the drosophilid stocks at the *Drosophila* Species Resource Center in Austin, Texas, USA. The second hypothesis was that the introduction occurred directly through the air transport of contaminated foods from Africa to São Paulo.

A third hypothesis, proposed by Galego and Carareto (2007) as part of an analysis of esterase polymorphism, was that the introduction occurred through maritime transport in the Port of Santos (this port is a likely site, considering its commercial importance for Brazil: one quarter of all the products imported by Brazil passes through this port). According to the Food and Agriculture Organization (FAO, 1997) the global fruit market increased by 13% in the period 1985-1995. The volume of fruit transported and the special requirements for preservation mean that most of the fruit trade involves transport by sea (França and Gondin, 1999). According to data from the Brazilian Institute of Fruits (IBRAF, 2000-2001), Brazil imports mainly apples, cherries, grapes, kiwi, nectarines, peaches, pears and plums. Brazilian imports showed a significant increase

from the 1970s to the 1990s, including imports from the African continent. Brazil currently has bilateral trade agreements with most countries in Africa (Ministério de Relações Exteriores, 2007), especially South Africa, from where it imports ores and agricultural products, including grapes (Ministério do Desenvolvimento, 2007). The importance of the fruit trade for the dissemination of this species in Brazil was initially suggested by Tidon *et al.* (2003). Galego and Carareto (2007) suggested that after its introduction, *Z. indianus* spread throughout the state of São Paulo principally via the highway-based fruit trade.

The second and third hypotheses are the most likely because data from morphological, ecological and genetic markers indicate that the founding population was quite large (David *et al.*, 2006a; Ananina *et al.*, 2007; Galego and Carareto, 2007). However, regardless of how it was introduced, *Z. indianus* rapidly expanded its range; in little more than two years after its introduction, this drosophilid was present in practically all of the Brazilian states. As early as 1999, after the first report, the species was detected in Santa Catarina (Toni *et al.*, 2001) and other areas of the Brazilian cerrado and Midwest (Tidon *et al.*, 2003). In 2000, the species reached Rio Grande do Sul (Castro and Valente, 2001), Rio de Janeiro (Loh and Bitner-Mathé, 2005) and Uruguay (Goñi *et al.*, 2001, 2002). In 2001, the species was recorded in different locations in Minas Gerais (Kato *et al.*, 2004; David *et al.*, 2006a), and in 2002 it was found in various states in the Northeast (Mattos-Machado *et al.*, 2005). The species reached the state of Tocantins and the north of Brazil in 2003 (Santos *et al.*, 2003) and was also recorded in Panama in 2003 (Central America). In 2005, *Z. indianus* was recorded in Florida (USA) (van der Linde *et al.*, 2006) and Argentina (Soto *et al.*, 2006). This rapid and broad geographic dispersion is indicative of the great ease with which *Z. indianus* can colonize new environments. Figure 1 shows the worldwide distribution of *Z. indianus* (with Brazil highlighted) and the probable dates of colonization.

Interest in the study of *Z. indianus* is directly related to its recent invasion of Neotropical regions. Today, this species is considered to be semi-cosmopolitan (Vilela, 1999; Tidon *et al.*, 2003; Silva *et al.*, 2005a,b). This rapid expansion, in addition to the scarcity of information on *Z. indianus* until the last ten years, has motivated dozens of researchers to investigate the invasion by this species. Many of the studies that have investigated this invasion have used markers such as quantitative characteristics, alloenzyme polymorphisms, mitochondrial DNA, ecological analysis and even complete genome sequencing.

Variation in quantitative traits can be a good indicator of the amount of genetic variability in a species and can reveal the potential phenotypic plasticity of the species and its ability to exploit niches. Loh and Bitner-Mathé (2005) detected significant variation in wing size and form in populations of *Z. indianus* in Rio de Janeiro. David *et al.* (2006a) analyzed three quantitative characteristics (wing

Figure 1 - Migration routes for *Z. indianus* involved in its dispersal throughout the world, based on studies cited in the text. The process that occurred in Brazil is highlighted.

size, thorax size and number of sternopleural bristles) in African, Indian and Brazilian populations of *Z. indianus* and found clinal variation in the Indian populations that was less marked in the African populations and not detected in the Brazilian populations; however, the Brazilian populations showed significant interlineage differences. Based on these data, the authors suggested that the propagules that colonized Brazil were quite numerous and contained sufficient genetic variability to prevent a possible "bottleneck" effect. The authors also suggested that the colonization was quite recent, which would explain the lack of clinal variation in the quantitative traits. Analysis of the average body size of Brazilian *Z. indianus* suggested that South Africa was the probable origin of the founder propagules (David *et al.*, 2006a). This conclusion regarding the African origin of the Brazilian populations was supported by the lack of significant differences between populations from these two geographic regions (David *et al.*, 2006a). Chromosomal inversions also support the hypothesis of an African origin for the founder propagules of *Z. indianus* (Ananina *et al.*, 2007). Only inversion In(II)A, one of the most frequent in Indian populations (Gupta and Kumar, 1987), was detected in Brazilian populations, but at a very low frequency. Ananina *et al.* (2007) also showed

that Brazilian populations contained five other inversions not detected in Indian populations. The high rate of inversion polymorphisms, along with the fact that they are rarely shared with Indian populations, indicates that the founder propagules were quite numerous and that they did not stem from the latter populations.

In various drosophilids, the colonization of a new environment leads to an increase in genome size through enhanced transposition of transposable elements (Biemont and Vieira, 2005). The size of the *Z. indianus* genome as estimated by flow cytometry ranges from 0.601 pg in Indian populations to 0.630 pg in African populations and 0.635 pg in Brazilian populations (Nardon *et al.*, 2005). According to these authors the smaller genome size of Indian populations suggests a possible Asiatic origin for this drosophilid. Among the different markers studied to date, genome size is the only one that suggests a non-African origin for *Z. indianus*; however, the smaller genome size of the Indian populations may be the results of a recent bottleneck during occupation of the Palearctic region (Yassin *et al.*, 2008b). At any rate, these data indicate that the Brazilian and African populations have a similar genome size, which again supports the idea that the founding Brazilian populations were of African origin.

Molecular markers have contributed considerably to understanding the introduction of *Z. indianus* into the Americas. Esterase alloenzymes were the first markers to be used. 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. This polymorphism supports the hypothesis that South America was colonized by a large number of propagules. Mattos-Machado *et al.* (2005) also analyzed polymorphisms at five alloenzyme loci (Acp, Pgm, Idh, Hk and Est-3) in Brazilian, Asiatic and African populations of *Z. indianus* and detected a low F_{ST} among the Brazilian populations, which suggested colonization by a single propagule with subsequent rapid expansion. Although these authors did not suggest the origin of the propagule, they stated that it probably included almost all of the polymorphisms that existed in the ancestral population. An African origin for *Z. indianus* is also supported by the analysis of neutral polymorphisms, which are more appropriate for phylogeographic studies than alloenzymes, such as the mitochondrial genes COI and COII (Yassin *et al.*, 2008b; Commar, Ceron, Carareto, unpublished data). Additionally, it has been also reinforced by analysis of the nuclear gene β esterase-6 (Commar, Ceron, Carareto, unpublished data).

Biological characteristics of *Zaprionus* indianus related to colonization in the Neotropical region

Zaprionus indianus is a generalist species that uses a variety of domestic and non-domestic fruits as sites for mating and oviposition (Lachaise and Tsacas 1983;

Schmitz *et al.*, 2007). In Brazil, *Z. indianus* has adopted a behavior never seen before among drosophilids, namely, the colonization of unripe fruits, making them inedible to humans and causing extensive economic damage (Castro and Valente, 2001). Although not considered a pest in its place of origin, the invasion of Brazil by this drosophilid has resulted in considerable agricultural losses. In 1999, *Z. indianus* was responsible for the loss of 40% of the fig harvest (*Ficus carica*) in the main productive region in the state of São Paulo (Stein *et al.* 2000). As a result, *Z. indianus* was classified as a pest at the time; however, this behavior must have been a single, one-off event that characterized the introduction of *Z. indianus* into Brazil (van der Linde *et al.*, 2006).

Some characteristics of *Z. indianus*, such as its variation in body size, are similar to those of populations found on other continents (Yassin *et al.*, 2009b). Body size may be related to the success of invading species (Cassey, 2000; Roy *et al.*, 2002; Fisher and Owens, 2004). The variability in fly size in a population of *Z. indianus* in the Nile delta, which has a Mediterranean climate, was initially attributed to the highly heterogeneous environment of this region, including high temperatures, stress, dehydration and exposure to insecticides (Yassin *et al.*, 2007). Yassin *et al.* (2009b) examined this hypothesis by investigating other populations of the same species living in a completely different and more benign tropical environment, such as close to the tropics in Brazil, where the climate is wet and humid. The populations sampled were genetically different from the Egyptian population, as shown by cytogenetic (Ananina *et al.*, 2007) and molecular (Yassin *et al.*, 2008b) studies. The authors showed that contrary to expectation, body size variability was always very high and similar across populations and continents. These results suggested that the elevated phenotypic variability in *Z. indianus* may be an intrinsic property of this species and may be related to the ability to use a wide diversity of resources and micro-habitats.

Other studies that have examined the invasive potential of *Z. indianus* have focused on the life cycle (Amoudi *et al.*, 1991), larval competition (Amoudi *et al.*, 1993a) and fitness components (Amoudi *et al.*, 1993b) in lineages originating in Saudi Arabia. *Zaprionus indianus* is a tropical species that is easily reared at 31 °C (Amoudi *et al.*, 1991; Karan *et al.*, 1999; Araripe *et al.*, 2004; Loh *et al.*, 2008) but is sensitive to cold. The optimal temperature for successful development of flies from Saudi Arabia is 20-30 °C, with no development at 35 °C (Amoudi *et al.*, 1991, 1993b). This sensitivity to variation in temperature is an important factor in the establishment of this species in varied environments such as Saudi Arabia, where elevated temperatures are frequent during the summer.

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 in-

secticides and selection stemming from climatic variation (Galego and Carareto, 2007, 2010b). Plasticity in the distribution of allele frequencies for the Est-3 locus may also have contributed to the successful spread of this organism, especially in the American continent, given that esterases perform multiple essential functions in insects.

Until the end of the 1990s, few studies had examined the life cycle of *Z. indianus.* Stein *et al.* (2003) and Setta and Carareto (2005) contributed significantly to our understanding of the life cycle of *Z. indianus* populations. These studies reported a greater longevity than in *Z. indianus* populations of Indian (Bains *et al.*, 1995, 1996) and Saudi Arabian (Amoudi *et al.*, 1991, 1993a) origin. The productivity of the species was similar to or greater than that of other drosophilids and the development time was very similar to that of *D. sturtevanti.* These results indicated an *r*-strategy of environment colonization, which is highly characteristic of bioinvaders.

Fitness components, such as development time, productivity and fertile period are strongly linked to the reproductive biology of a species, with fecundity and productivity being directly related to the production of ova and sperm. Araripe *et al.* (2004) demonstrated that the viability of male gametes in *Z. indianus* was temperature-dependent, such that very low ambient temperatures led to male sterility. If development occurred at 15 °C, all of the males were sterile. This drastic reduction in reproductive capacity could explain why *Z. indianus* is not found in higher latitude regions, as already reported by Chassagnard and Kraaijeveld (1991) and Goñi *et al.* (2001, 2002).

We do not know how the species survives periods of cold, *i.e.*, if there is diapause or if populations are able to recover through reintroduction. One hypothesis suggested by Danni (1980) involves the formation of islands of heat in cold regions, as for example in the southern Brazilian city of Porto Alegre. This phenomenon is associated with urbanization. These thermal islands could be used as refuges by urban insect populations during unfavorable periods. Oscillations in the frequencies of different species reflect variations in their tolerance to variable climatic conditions at a single location. According to Tidon (2006), some drosophilid species are extremely seasonal and only appear at particular times of the year. In agreement with this, the highest frequencies of *Z. indianus* were recorded during the seasons with the highest average temperatures (spring and summer), whereas sightings were lowest during the fall and winter but increased again in the spring. Such fluctuations reinforce the invasive capacity of this species (Tidon *et al.*, 2003). Similar behavior was observed by Silva *et al.* (2005a) in three urban parks of Porto Alegre over seven seasons. In this case, *Z. indianus* showed the highest frequencies compared to other drosophilids during the seasons with the highest mean temperatures, but the frequency consistently dropped during autumn and winter to increase again in the spring. The authors concluded that the ability to

live in environments associated with humans and the capacity to restore high population levels under favorable conditions contributed to this species expansion and colonization of new areas.

The distribution of *Z. indianus* across different continents and its establishment in these areas is related to the climatic conditions that it encounters. Mata *et al.* (2010) used multivariate analysis to show that *Z. indianus* occupies different niches in Africa, Asia and the Americas such that the climatic conditions of the area occupied by the original population differ from those of areas where it is new, principally in India. Indeed, populations of *Z. indianus* in India established themselves in climates very different from those of Africa, where the temperatures are more variable and considerably lower in the colder months. The clinal variation in several characteristics of the Indian populations of *Z. indianus* (Karan *et al.*, 2000) may reflect the adaptation of these flies to these conditions. Changes in niche can occur through adaptive responses to new environmental conditions in the invaded areas and these changes may be driven by natural selection for climatic tolerance (Mata *et al.*, 2010). In South America, invading *Z. indianus* encountered climatic conditions very similar to their original niche that allowed the rapid establishment and expansion of this species throughout Brazil.

Studies on the distribution of *Z. indianus* in Brazil have shown variation in the abundance of this species among ecosystems. Tidon *et al.* (2003) analyzed the abundance of *Z. indianus* in the cerrado and riverine forests and found a greater abundance in the cerrado during wet periods. Ferreira and Tidon (2005) showed that, together with *D. simulans*, *Z. indianus* was the most abundant species in the urbanized environment of Brasília, the Brazilian capital. On the other hand, the abundance of this fly in mangrove forests was higher than in the Atlantic rain forest but lower than in the cerrado (Tidon *et al.*, 2003) or in urban environments (Ferreira and Tidon 2005; Silva *et al.*, 2005b). These results indicate that together with other introduced Drosophilidae, *Z. indianus* could be useful as an indicator of disturbed areas.

In addition to the factors discussed above, the variation in *Z. indianus* abundance may also reflect competitive interactions with other drosophilids. An experimental study has shown a reduction in the viability of *Z. indianus* in the presence of larval waste from *D. sturtevanti*; on the other hand, waste from *Z. indianus* interferes with the viability of *D. simulans* and the duration of development in both of these *Drosophila* species (Galego and Carareto, 2007). Thus, competitive interactions between *Z. indianus* and other drosophilids may affect the population density of this species after its introduction into a new environment. Other factors, such as temperature tolerance and plasticity in the occupation of niches (involving the use of a wide variety of plants as food sources) may also be related to this species invasive success.

Acknowledgments

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