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# Cactophilic *Drosophila* in South America: a model for evolutionary studies

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**Abstract**

The *Drosophila buzzatii* cluster is composed of seven cactophilic species and their known geographical distribution encompasses the open vegetation diagonal, which includes the morphoclimatic Domains of the Caatinga, Chaco and Cerrado, which are situated between the Amazon and the Atlantic forests. Besides these areas, these cactophilic species are also found in a narrow strip along the Atlantic coast from northeastern Brazil to the southern tip of the country. The hypothesis of vicariant events, defining the core areas of each species, is proposed to explain the historical diversification for the cluster. The intraspecific analysis for the cluster shows a population structure with gene flow restricted by distance, range expansion with secondary contact resulting in introgression and simpatry, especially in the limits of the species distribution, polytypic populations and assortative mating in inter population experiments. There is a variation related to these events that depends on the species and geographic origin of the population analyzed. These events are, hypothetically, described as the results of expansion and retraction of the population ranges, as a consequence of their association with cacti, which theoretically follow the expansion and retraction of dry areas during the paleoclimatic oscillations in South America, as that promoted by the glacial cycles of the Quaternary. The *Drosophila buzzatii* cluster is divided into two groups. The first one is composed of *D. buzzatii*, a species that has a broad geographic distribution and no significant differentiation between its populations. The second is the *Drosophila serido* sibling set, which encompasses the others species and is characterized by a significant potential for differentiation.

## Introduction

In South America, there is a large area, oriented along a northeastern-southwestern axis, called the 'dry diagonal' (Prado & Gibbs, 1993), composed of xerophitic vegetation and located between the Amazon and the Atlantic tropical rain forests. This corridor includes the Caatinga Domain in the northeast of Brazil, the Chaco Domain that extends throughout a great part of the Argentinean territory east of the Andes, southern Bolivia, western and central Paraguay and a narrow strip west of the state of Mato Grosso, in Brazil. Both formations are conditioned by a semiarid climate, and the Cerrado Domain savannah like, with a physiognomy varying from closed forests to open fields, extends throughout the plains of central Brazil.

The morphoclimatic domains of the Caatinga and Chaco have a high density and diversity of cacti species as the Caribbean coasts of Colombia and Venezuela (Hueck, 1972). Adjacent domains, including forests, also contain cacti as isolated populations in rocky leveling or associated with vegetation formations in sandy substrates. These cacti populations are thought to be reminiscent of the retraction of the xeromorphic vegetation in interglacial periods. In South America, during the

last glacial period (approximately 13,000–18,000 years ago), there was an expansion of the open formations over large forest masses; these changes are also tied to the conditions of the topographic detail of the Brazilian plains and of the central depressions of South America, as well as various small centers and higher altitude peri-glacial areas of the Andes mountains (Ab'Saber, 1977, 1992; Vanzolini, 1981). According to Ab'Saber (1977), the Caatinga and the Chaco domain were connected during the Pleistocene Era. However, based on floristic composition, Prado and Gibbs (1993) suggested that these two domains are not historically connected because the Chaco domain is derived from dry vegetation from the southern extreme of South America.

The occurrence of the cacti in the American continent remounts to approximately 100 million years. Outside of the Americas, only cacti of the *Rhipsalis* genus occur on Madagascar Island (Brown & Gibson, 1983). If the cacti followed the expansion and retraction of the distribution of the dry areas in the paleo environments they may have been important in the differentiation processes of the species and organisms associated with them as, for example, the *Drosophila* species of the *Drosophila buzzatii* cluster that utilize decaying cacti as an exclusive feeding resource for its larvae (Pereira, Vilela & Sene, 1983).

## The *Drosophila buzzatii* cluster

The *Drosophila buzzatii* cluster (*repleta* group, *Drosophila* genus) is today composed of seven nominal species: *D. buzzatii* (Patterson & Wheeler, 1942), *D. serido* (Vilela & Sene, 1977), *D. antonietae* (Tidon-Sklorz & Sene, 2001), *D. seriema* (Tidon-Sklorz & Sene, 1995a), *D. gouveai* (Tidon-Sklorz & Sene, 2001), *D. borborema* (Vilela & Sene, 1977) and *D. koepferae* (Fontdevila & Wasserman, 1988). With the exception of *D. buzzatii*, which was introduced to other continents along with the *Opuntia ficus-indica*, one of its host cacti (Barker et al., 1985), all of these species are endemic to South America (Figure 1). The *D. buzzatii* cluster, with the *D. stalkeri* and *D. martensis* clusters, belongs to the *Drosophila buzzatii* complex which is connected to the other species of *repleta* group by sharing a chromosomal arrangement denominated PRIMITIVE I (Ruiz & Wasserman, 1993). For the *D. buzzatii* complex, a parsimony analysis, using data from chromosomal inversions, suggests that the *D. stalkeri* cluster occurring in the Caribbean and in Florida is the most primitive; the *D. martensis* cluster, occurring in Venezuela and Colombia, is the most derivate group and the *D. buzzatii* cluster, found in Brazil, Argentina and Bolivia, is the intermediate one (Ruiz & Wasserman, 1993).

The *D. buzzatii* cluster association with decaying tissues of cactus results in at least two consequences. The first is that the present distribution of its populations is determined by the occurrence of Cactaceae in South America, which includes the Caatinga, Chaco and, between these areas, a great number of isolated populations, which are more restricted in size. The second consequence is that the differentiation processes within this cluster can be related to the expansion and retraction of the open vegetation in South America during climatic changes, such as those promoted by the glacial periods (Sene, Pereira & Vilela, 1988).

In the 70s and 80s, with the exception of the *D. buzzatii*, all flies from the known collecting areas belonging to the *D. buzzatii* cluster were identified as *D. serido* or as *D. borborema* (Vilela & Sene, 1977; Vilela, Pereira & Sene, 1983). All species from the *D. buzzatii* cluster have a very similar external morphology, but are easily identified by aedeagus since they show distinct shapes and sizes (Vilela & Sene, 1977). However, the population analyses of *D. serido*, which occurs from the northeast of Brazil to the Chaco Domain, using different markers, such as the aedeagus morphology (Silva & Sene, 1991), the presence of paracentric chromosomal inversions (Tosi & Sene, 1989), metaphase karyotype (Baimai, Sene & Pereira, 1983) and degree of reproductive isolation (Bizzo, 1983), showed that it was a polytypic species (Sene, Pereira & Vilela, 1982, 1988). Regarding to the aedeagus morphology, Silva & Sene (1991) observed the existence of five morphotypes in *D. serido*, denominated by the letters A–E, which are very similar in shape, with quantitative differences between them. For the characterization of these structures and identification of the species, the aedeagus were divided into four main regions delimited by reference marks denominated 'the markpoints' (Silva & Sene, 1991) (Figure 2). Each region delimited by adjacent markpoints describes a region denominated arch. Based on the length of

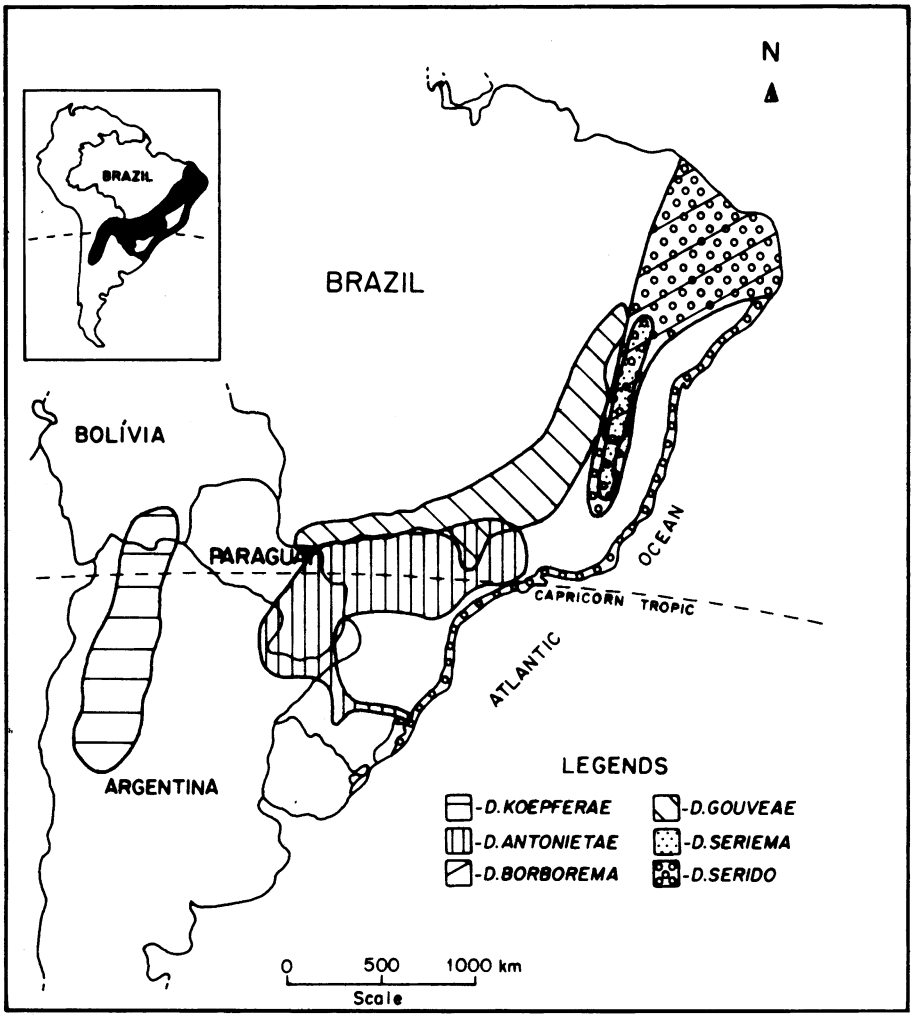


*Figure 1.* The known geographic distribution of the species belonging to *Drosophila buzzatii* cluster. The *D. buzzatii* species is not marked on the map because it occurs in all areas of the species of the *D. serido* sibling set.

these arches, five distinct morphotypes of aedeagus were discriminated. Arches II and III, in the superior distal portion, are the most informative for the discrimination of the morphotypes. Later, four morphotypes of *D. serido* were elevated to the category of species due to the concordance among the various markers in most of the populations. These four new species (*D. koepferae, D. seriema*, *D. gouveai* and *D. antonietae*) with *D. serido* and *D. borborema*, previously described, came to be the '*D. serido* sibling set' (Tidon-Sklorz & Sene, 2001), replacing the designation of '*Drosophila serido* superespecie taxon' proposed by Fontdevila et al. (1988) because this species group does not fit the definition of 'superespecie' according to Mayr (1963). Thus, the *D. buzzatii* cluster is composed of the *D. buzzatii* and the six species of the '*Drosophila serido* sibling set'. The aedeagus is the main diagnostic character to identify all of these species (Silva & Sene, 1991; Tidon-Sklorz & Sene 1995b).

## Drosophila koepferae

In the *D. buzzatii* cluster, the populations identified as morphotype E of aedeagus, endemic to the Chaco Domain (Silva & Sene, 1991), were described as *D. koepferae* (Figure 1). This species is characterized by the fixed chromosomal inversion $2j^9$ (Wasserman & Richardson, 1987)

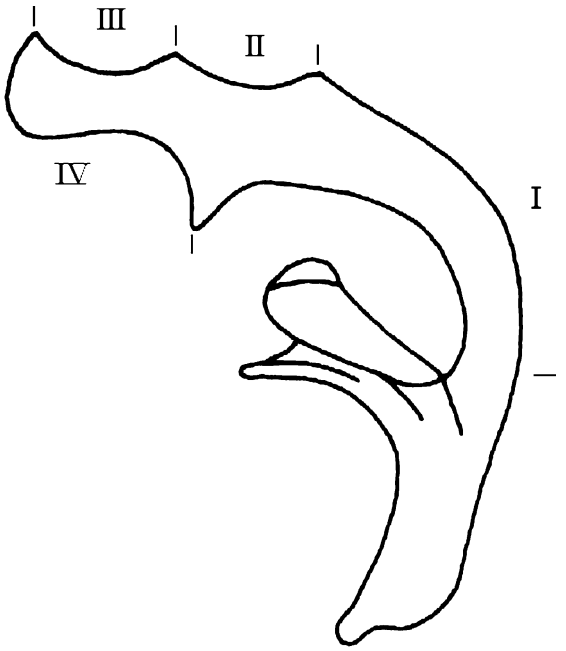


*Figure 2.* Lateral right view of the aedeagus outline of *D. serido* sibling set. I–IV are the arc regions defined by the mark-points: (I) anterior region of the right dorsal margin; (II) fused region between the left and right dorsal margins; (III) posterior region of dorsal margin; (IV) posterior region of the ventral margin (Silva & Sene, 1991).

and the karyotype VI (Baimai, Sene & Pereira, 1983) (Figure 3). With regard to the population structure for *D. koepferae*, there is little information. An incipient racial differentiation among the populations of Argentina and Bolivia was described by the analysis of polymorphic chromosomal inversions and the genetic distance calculated from the allelic variation of protein (Fontdevila et al., 1988). The ethological isolation among the populations of Argentina of *D. koepferae* and the populations of the *D. serido* species is complete, while the populations of Bolivia are only partially isolated from *D. serido* (Fontdevila et al., 1988).

Considering the morphological aspects of aedeagus of the species of the *D. buzzatii* cluster, *D. koepferae* is part of the *Drosophila serido* sibling set. However, *D. koepferae* and *D. buzzatii* share the tergite pattern and are lighter in color when compared with other *D. buzzatii* cluster species (Fontdevila et al., 1988). Only the analysis using mtDNA, a cytoplasmatic marker, allocated *D. koepferae* as phylogenetically close to *D. buzzatti* species (Manfrin, DeBrito & Sene, 2001). All of the other analyses using nuclear markers, such as a nucleotide sequence of *Xdh* (Rodrigues-Trelles, Alarcon & Fontdevila, 2000), satDNA (Kuhn & Sene, 2005) and the wing morphology (Moraes et al., 2004) allocated *D. koepferae* into the *Drosophila serido* sibling set species (Figure 4).

Drosophila seriema

Tidon-Sklorz & Sene (1995a) described the populations having morphotype C of aedeagus as *Drosophila seriema* (Figure 1). The analyzed populations of this species have the fixed chromosomal inversion $2e^8$ (Tosi & Sene, 1989) and karyotype II (Baimai, Sene & Pereira, 1983) (Figure 3), although different karyotypes were described in two populations in the northern part of its geographic distribution, due to different amounts of heterochromatin in chromosome six (Kuhn et al., 1996). This species is endemic to the Espinhaço mountain range, occurring at altitudes above 1000 m, where open vegetation called 'campo rupestre' (rocky fields) predominates (Giulietti et al., 1987), with a great variety of cactus since these regions are endemic areas for the species of the *Pilosocereus* genus and many other groups of insects and plants (Giulietti et al., 1987; Zapi, 1994). The geographical distribution of this species suggests that its isolated populations are structured as the islands model (Tidon-Sklorz & Sene, 1995a). The geographical variation analysis showed only one polymorphic chromosome inversion (Tosi & Sene, 1989), one basic karyotype denominated type II (Kuhn et al., 1996), no significant variation in aedeagus morphology (Tidon-Sklorz & Sene, 1995a) and a high inter-population homogeneity by the analysis of a satDNA family (Kuhn & Sene, 2004). These data sets suggest that this species is a monomorphic evolutionary lineage. Based on these data, the authors suggested a recent origin for this species or the existence of gene flow among its populations. This low level of variability is counter to the information obtained from the analysis of its populations through the haplotypic variation of mtDNA, that showed divergence among its populations, with levels similar to those inter-specifically obtained for the other species of the *D. buzzatii*, suggesting this species to be paraphyletic (Manfrin, DeBrito & Sene, 2001). If the hypothesis of the recent

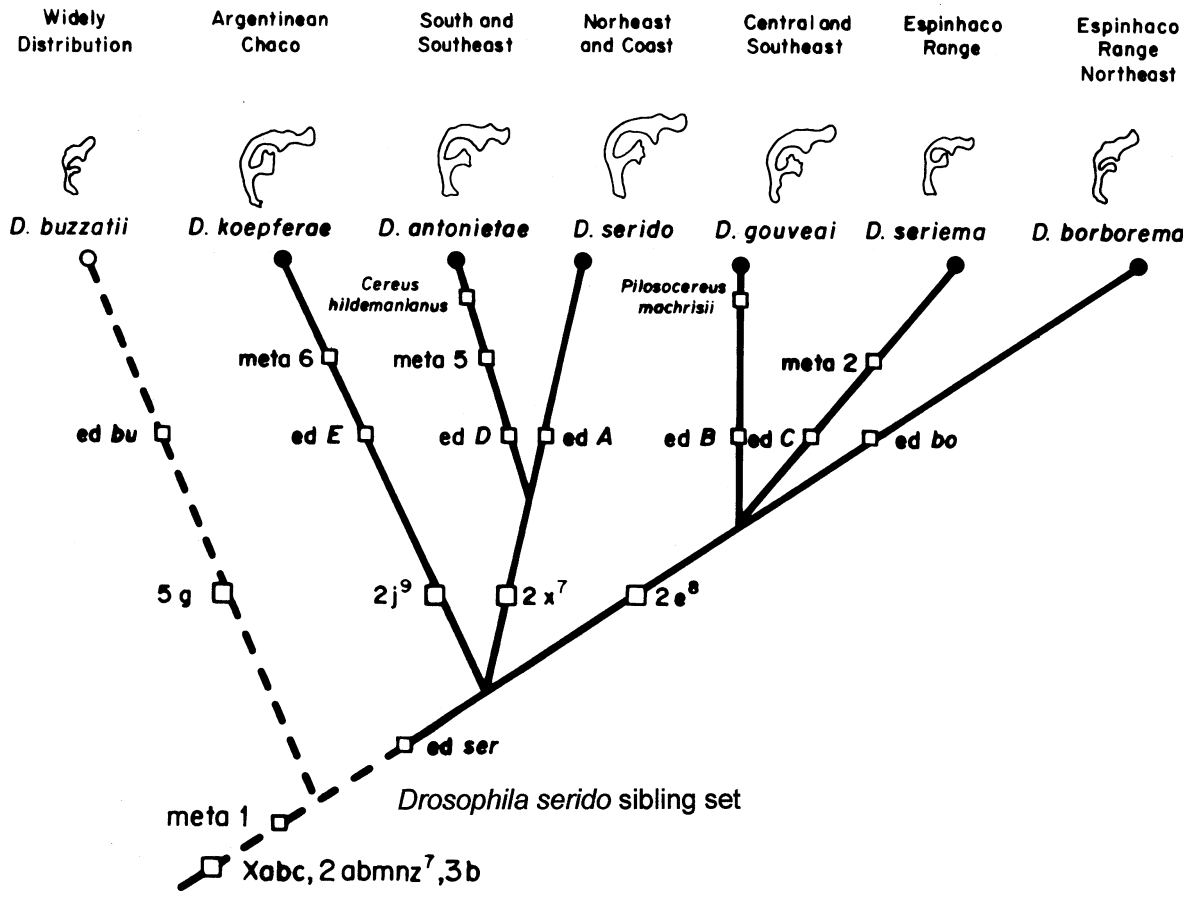


*Figure 3*. Phylogenetic tree of the *D. buzzatii* cluster, primarily based on chromosome inversions (Wasserman & Richardson, 1987; Tosi & Sene, 1989; Ruiz & Wasserman, 1993; Ruiz et al., 2000); ed = A–E are the aedeagi morphological types (Silva & Sene, 1991), bu = *D. buzzatii* aedeagus and bo = *D. borborema* aedeagus; meta = metaphasic chromosome types (Baimai, Sene & Pereira, 1983). The full lines sort the species of the *D. serido* sibling set, after Tidon-Sklorz & Sene (1995b).

origin is indeed correct, the analysis of the mtDNA variation may be the result of the ancestral polymorphism or the result of a restricted gene flow among the populations. Nevertheless, another possibility that can be considered is events of differential introgression of mitochondrial haplotypes as a result of hybridization with other species of the cluster. These events could be possible because *D. seriema* is sympatric with other species of the *D. buzzatii* cluster throughout its distribution (Figure 1). Experiments of hybridization, in laboratory, among species of the *D. buzzatii* cluster have shown different degrees of reproductive isolation with no cases of complete isolation (Bizzo, 1983; Marin et al., 1994; Madi-Ravazzi, Bicudo & Manzato, 1997).

Drosophila gouveai

*Drosophila gouveai* includes the populations of the B morphotype of the aedeagus (Tidon-Sklorz & Sene 2001). This species is characterized by the fixed chromosome inversion $2e^8$ (Tosi & Sene, 1989) and by karyotype I (Baimai, Sene & Pereira, 1983) (Figure 3). It has a large area of geographic distribution, occurring in the mid-west region of Brazil, lying to the west of the Espinhaço mountain range (Figure 1), associated with the 'campo rupestre' vegetation that reaches the southern extreme of the cerrado vegetation distribution in Brazil (Tidon-Sklorz & Sene, 2001).

The distribution of the populations of this species can be characterized in two ways. The

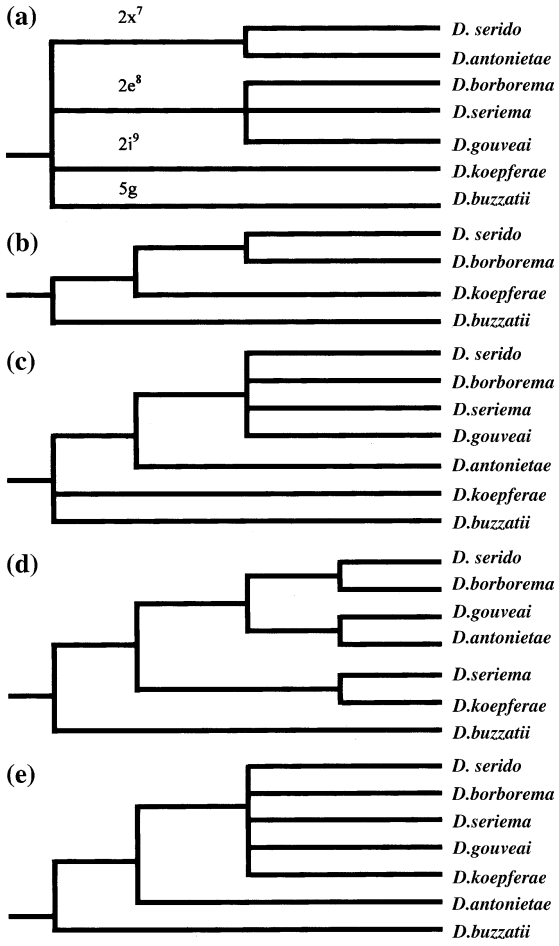


*Figure 4*. Phylogenetic hypotheses of the *D. buzzatii* cluster based on different data sets: (a) based on fixed chromosomal inversions, after Ruiz et al. (2000); (b) based on the *Xdh* nucleotide sequences, after Rodriguez-Trelles, Alarcon and Fontdevila (2000); (c) based on the mitochondrial COI sequences, after Manfrin, DeBrito and Sene (2001); (d) based on morphological distances from 17 wing parameters, after Moraes et al. (2004); (e) based on satellite DNA, after Kuhn (2003).

populations from the southern portion of its distribution area are found in sandstone table hills with 'campo rupestre' vegetation, associated with the *Pilosocereus machrisis*, forming a pool of isolated populations (Monteiro & Sene, 1995; Tidon-Sklorz & Sene, 2001; Moraes & Sene, 2002, 2003). In the north and northeast portion of its distribution, its populations occur in large areas with a variety of cacti species such as the *Pilosocereus* and the *Cereus* (Pereira, Vilela & Sene, 1983). The aedeagus morphology analysis of populations from the southern limit of its distribution showed that each of these populations could be discriminated, suggesting isolation among them (Monteiro & Sene, 1995). These data have led to the elaboration of the hypothesis in which the structure of these populations would be in accordance with the islands model that would allow the acquisition of the differentiation as that observed in the morphology of the aedeagus.

Moraes and Sene (2002) have shown that besides the populational geographic structure, this species presents, in one of its populations, an organization related to the availability of its host cactus, forming intrapopulational endogamic groups. The cactus in this locality is *Pilosocereus machriisis*, which is a thin columnar cactus, and its decaying tissue remains for only a few days. Therefore, each decaying cladode would serve as a substrate for only one generation of flies. This work also indicated a tendency in individuals of *Drosophila* to disperse only to find a decaying cactus, as has already been shown in works conducted with other cacti species (Johnston & Templeton, 1982; Markow & Castrezana, 2000). This population is theoretically isolated on a sandstone table hill and does not receive genetic material through the gene flow from other populations. This fact, associated with the structure of inbreeding detected, could be responsible for a reduction in the genetic variation of the population. Nevertheless, this reduction is not observed in this population, whose levels of variability are similar to other species populations and whose ecological and demographic characteristics are distinct (Moraes & Sene, 2002).

In regard to the inference of the historic events, which explain the present distribution of *D. gouveai*, the analysis of the haplotype distribution of the mtDNA COI gene has shown that its populations are structured. This species within the *D. buzzatii* cluster belongs to the clade composed by the *D. serido*, *D. seriema* and *D. borborema* species. The oldest mitochondrial haplotypes in this clade are located in the northeast of Brazil, which suggests that the northeastern region is the place of origin of the clade. Thus, from the northeast, the known distribution of *D. gouveai* is the result of at least two events of range expansions reaching to the central and southern regions of Brazil (Manfrin, DeBrito & Sene, 2001; DeBrito, Manfrin & Sene, 2002a).

Phenetic analysis, using wing morphology and the allelic frequency variation of microsatellite loci, indicates a fragmentation separating the *D. gouveai* populations into three groups: a group in the north, another group in the west region and a third one encompassing the populations of the southern distribution of the species (Moraes et al., 2004). The author, based on the distribution and level of genetic variation, suggests this geographical structure is a result of historical association among populations within regions rather than ongoing gene flow.

The interesting fact about the two analyses previously mentioned is that the data for *D. gouveai* show, regardless of the methodology and marker used, almost the same population substructure. The distribution can be explained as the result of events of independent colonization in the west and south, from the north of the distribution of the species, as suggested by the analysis of the mtDNA (DeBrito, Manfrin & Sene, 2002a) and maintenance of the differences that characterize these populations through the restricted gene flow by distance (Moraes et al., 2004).

The *D. gouveai* populations at the southern limit of their distribution are involved in different evolutionary events. The analysis of individuals from populations of Rifaina, Brotas and Analândia localities (the southern limits of species distribution) showed that these populations have aedeagus morphology, host plant, chromosome inversion and karyotype data, which indicate that they belong to *D. gouveai* species. However, the mitochondrial haplotype variation (Manfrin, DeBrito & Sene, 2001; DeBrito, Manfrin & Sene, 2002a) has allocated the analyzed individuals from these populations in the clade composed by populations of the *D. antonietae* species. This data showed a lack of agreement between the genealogy of COI haplotypes and the evolutionary relationships of the individuals. This fact can be explained, by among other possible causes, as an event of secondary contact between populations of these two species due to a populational range expansion during glacial periods. The *D. gouveai* populations analyzed are located at the southern end of the species geographical distribution, which is also the northern limit of the distribution for *D. antonietae* species. In this region, populations of *Cereus hildmaniannus* and *Pilosocereus machrisis* cacti have grown close together inside a east–west corridor of approximately 200 km, geologically formed by the limits of sandstone and basalt formations with limestone outcrops, seemingly the end of the geologic formation from the Canastra mountain range. This proximity suggests secondary contact between *D. gouveai* and *D. antonietae* at the limits of their distribution and differential introgression of mitochondrial genomes, in this case the unidirectional gene flow from the *D. antonietae* to *D. gouveai* (Manfrin, DeBrito & Sene, 2001; DeBrito, Manfrin & Sene, 2002a).

## Drosophila antonietae

Populations characterized by the aedeagus morphotype D were elevated to the category of species with the name *D. antonietae* (Tidon-Sklorz & Sene, 2001). This species has the fixed chromosome inversion $2x^7$ (Ruiz et al., 2000) and the karyotype V (Baimai, Sene & Pereira, 1983) (Figure 3). It is found in southern and southeastern regions of Brazil and along northern border of the eastern part of the Chaco Domain (Silva & Sene, 1991) (Figure 1). This region corresponds to the basin of Parana, Paraguay and Uruguay Rivers, which constitutes a zoogeographic region that is an endemic area for a variety of species of Diptera and plants (Amorim & Pires, 1996; Oliveira-Filho & Ratter, 1995; Burnham & Graham, 1999). This region is the area of occurrence of the columnar cacti *Cereus hildmaniannus*, the host plant for oviposition of *D. antonietae* (Pereira, Vilela & Sene, 1983; Ruiz et al., 2000).

The populations of the *Drosophila antonietae* were extensively studied through the aedeagus morphology (Monteiro & Sene, 1995) and electrophoretic variability of alloenzymes (Mateus et al., 2003). These data, in agreement, have shown a positive correlation between genetic distances and geographic distances in the north–south direction of the distribution of the species. Based on these results, the authors suggest the existence of gene flow between their populations limited by distance and stabilizing selection, both recurrent events associated with ecological conditions. The hypothesis of gene flow would be favored by the distribution of the host cacti of this species in this region. The populations of cacti in this region are associated with mesophytic forests, which follow through the valleys of the basin of the Paraná–Paraguay–Uruguay Rivers. This distribution would

theoretically provide a corridor of dispersion to the individuals of *D. antonietae*, which could lead to gene flow between them. The hypothesis of dispersion through corridors formed by cacti throughout the rivers basins, called ecological distance, was tested through a comparison between the morphological differences and the geographical and ecological distances (Monteiro & Sene, 1995). There was a significant association between morphological and geographical distances. This suggests that the more distant the populations are, in a straight line or alongside the rivers, the larger the morphological differences among their aedeagus.

Analysis of the haplotype diversity of the mtDNA COI gene (Manfrin, DeBrito & Sene, 2001; DeBrito, Manfrin & Sene, 2002a) suggested a population structure due to historical events in *D. antonietae*. Its populations were divided into two groups: the first one occurring in the northern part of the distribution of the species; the second in the southern reaches and along the coastline of the states of Rio Grande do Sul and Santa Catarina. This structure is in agreement with previous data from Monteiro & Sene (1995) and Mateus et al. (2003). The mtDNA analysis, did not discriminate between an event of past fragmentation and restricted gene flow with isolation by distance because samples of individuals from populations situated between the two regions were not analyzed. However, as neighboring populations of the sampled areas share mitochondrial haplotypes, it is possible that a continuum of populations of these two regions exists, each sharing their gene pool with neighboring populations. Nevertheless, when populations from the extremes of distribution of *D. antonietae* were sampled it was shown that they do not share any haplotype (DeBrito, Manfrin & Sene, 2002a); therefore, it can be concluded that there is no gene flow between them. Another significant event detected through the analysis of the variability of the mtDNA haplotypes was a range expansion of populations from the midlands of the state of Rio Grande do Sul toward the Atlantic coast (DeBrito, Manfrin & Sene, 2002a) and colonization of this region to the north (Morales, Manfrin & Sene, 2004). This expansion might have been favored by the presence of cacti throughout the 'riograndense' central depression in the state of Rio Grande do Sul extending to the Atlantic coastline. On the coastline, the event of expansion detected is in the northern direction following the populations of cacti throughout the dunes of the states of Rio Grande do Sul and Santa Catarina. If associated to geomorphological data, this expansion event was recent, because the origin of these coastal plains is recent and dates from the Cenozoic period; more precisely, its formation was directly affected by marine regressions and transgressions of the last 10,000 years (Villwock et al., 1986).

The *Drosophila antonietae*, despite being considered relatively basal within the *D. buzzatii* cluster (Manfrin, DeBrito & Sene, 2001; Kuhn & Sene, 2005), shows comparative homogeneity among its populations.

## Drosophila serido

The *Drosophila serido* species has the aedeagus morphotype A and occurs in the state of Minas Gerais and in all states that compose the northeastern region of Brazil, and along the Atlantic coast, from the state of Rio Grande do Norte up to the state of Santa Catarina (Silva & Sene, 1991; Ruiz et al., 2000) (Figure 1). Their populations have the fixed chromosome inversion $2x^7$ (Wasserman & Richardson, 1987) (Figure 3). The populations of this species can be divided into two groups according to karyotype (Baimai, Sene & Pereira, 1983), polymorphic chromosomal inversions (Tosi & Sene 1989; Ruiz et al., 2000) and mitochondrial haplotypes (Manfrin, DeBrito & Sene, 2001). The populations from the countryside of the Brazilian territory are homogeneous for the markers analyzed. Along the coast, nevertheless, the populations are polytypic for chromosomal inversions and for karyotypes. Although all the populations have the fixed chromosomal inversions $2x^7$, four polymorphic inversions are restricted to the Caatinga, in the northeast, and two fixed inversions are restricted to the southern and southeast coast (Wasserman & Richardson, 1987; Tosi & Sene 1989; Ruiz et al., 2000). In the arid region of Rio de Janeiro state, the populations present the karyotype III, whose difference with Type I (characteristic of Caatinga populations in the northeast) is the increase of constitutive heterochromatic in the dots and in chromosome Y. In the coastal region of São Paulo state, the karyo-

type IV has dots similar to those of Type III, but the Y chromosome is similar to Type I (Baimai, Sene & Pereira, 1983; Ruiz et al., 2000) and in the southern part of the distribution of this species, in populations of the state of Santa Catarina, another karyotype was found (Biffi et al., 2001).

The analysis of the mitochondrial haplotype variability divides the Atlantic coast populations into two groups. The first one occurs in the northern state of São Paulo towards the northeast, and the second one from southern São Paulo state up to Santa Catarina state (Morales, Manfrin & Sene, 2004). The distribution of these polytypical populations along the coast coincides with the geographic distribution of differentiated populations of other *Drosophila* species, such as *D. meridionalis* (Baimai, Sene & Pereira, 1983) and also coincides with endemism areas of other insect and vertebrate groups (Amorim & Pires, 1996). Associations of independent groups to zoogeographic areas suggest that historical events of geographical isolation determine the differentiation pattern of the populations.

As mentioned before, *D. serido* within the *D. buzzatii* cluster, belongs to the clade whose ancestral mitochondrial haplotypes lie in the northeast region of Brazil (DeBrito, Manfrin & Sene, 2002a). Considering this hypothesis, there are two possibilities to explain the expansion of the geographic area of *D. serido* throughout the Brazilian coast. The populations may have expanded their areas from the northeast towards the south, differentiating themselves later in isolated populations due to vicariant events. Another possibility is the independent colonization of regions along the coast from the countryside, especially the Serra do Espinhaço and the Serra da Mantiqueira, through the valleys of rivers that form corridors connecting the Brazilian countryside to the Atlantic coast with later local differentiation. In the preceding hypothesis, a gradual differentiation is expected, with an eventual cline, due to the north–south distribution of the populations, isolated in a narrow territorial strip between the sea and the Atlantic Forest. Nevertheless, the abrupt ruptures of either chromosomal or molecular marker distribution reinforce the second hypothesis. Intense studies are currently being conducted in this area to clarify this problem.

## Drosophila buzzatii

The *Drosophila buzzatii* species has a broad geographic distribution in South America and is found in territories such as Argentina, Bolivia, Paraguay and Brazil (Vilela, 1983; Vilela, Pereira & Sene, 1983; Figueiredo & Sene, 1992) (Figure 1). The most common host plants for this species are various prickly pear species, principally *Opuntia quimilo*, *Opuntia sulfurea* and *Opuntia monacantha* (*sin. O. vulgaris*) in Argentina (Hasson, Naveira & Fontdevila, 1992) and *Opuntia monacantha* and *Opuntia ficus-indica* in Brazil (Pereira, Vilela & Sene, 1983), although *D. buzzatii* has also emerged from other genus of cacti such as *Cereus* and *Trichocereus* in Argentina (Hasson, Naveira & Fontdevila, 1992) and *Cereus* and *Pilosocereus* in Brazil (Pereira, Vilela & Sene, 1983; Ruiz et al., 2000; DeBrito, Manfrin & Sene, 2002b). This species presents a high populational density in the Chaco domain; (Vilela, Sene & Pereira, 1980; Ruiz et al., 1982) however, its density decreases towards the northeast of Brazil (Vilela, Pereira & Sene, 1983; Figueiredo & Sene 1992; Tidon-Sklorz et al., 1995).

Baimai, Sene and Pereira (1983) analyzed metaphase chromosomes from populations of *D. buzzatii* in Argentina and Brazil and the results have shown that all the populations analyzed presented the same karyotype. A similar pattern was observed in the analysis of allozymes with no significant differences in the allelic frequency among Argentinean and Brazilian populations. However, there are two exceptions: the first one is a population from the Brazilian northeast, which is monomorphic for one locus, although this locus is polymorphic in all other populations of this species; the second is a southern coastal population of Brazil, which is polymorphic for three loci, that are monomorphic in other populations (Barker et al., 1985). The Chaco populations have 16 polymorphic inversions (Ruiz, Naveira & Fontdevila, 1984) and only two of them were present in the populations of the south of Brazil and none were found in the northeast of Brazil (Barker et al., 1985; Figueiredo & Sene, 1992). This group of information has drawn the hypothesis that the *D. buzzatii* species has originated in the Chaco domain and expanded its area of occurrence towards the northeast of Brazil (Carson & Wasserman, 1965; Figueiredo & Sene, 1992).

Two main hypotheses have been proposed for the dispersion of *D. buzzatii* in Brazil. The hypothesis of passive dispersion proposes that the species recently colonized Brazil following the introduction of one of its host cactus, *Opuntia ficus-indica*, by human activities; this colonization has most probably been associated with some bottlenecking (Sene, Pereira & Vilela, 1988). The alternative hypothesis proposes a natural expansion of the *D. buzzatii*, following the expansion of the cacti during climatic changes in the Quaternary period in South America (Barker et al., 1985).

A phylogeographic analysis was done, by using the mtDNA COI by DeBrito, Manfrin & Sene (2002b), to get a better understanding of the *D. buzzatii* population structure and to test the hypothesis to explain the distribution of this species throughout South America. The results showed that the *D. buzzatii* species presents low nucleotide diversities (DeBrito, Manfrin & Sene, 2002b). The same result was found in analysis of a satDNA family for these populations (Kuhn et al., 2003), showing no interpopulation differentiation. Nevertheless, the mitochondrial analysis showed that the Brazilian populations would be, on average, 50% more polymorphic than Argentinean populations (DeBrito, Manfrin & Sene, 2002b). The samples of the Chaco domain showed a lower level of polymorphism, followed by the sample from the southeast of Brazil, while the northeast and south of Brazil presented a higher mean number of nucleotide diversity, with the northeastern region of Brazil presenting a level of polymorphism almost two times as high as the second most polymorphic area (south of Brazil), even considering its small population sample. The phylogeografic analysis for *D. buzzatii* populations detected events of gene flow restricted by distance, in addition to an event of isolation by distance or vicariance separating the populations of the northeast of Brazil.

In fact, the observation of high diversity of nucleotides and the most divergent haplotypes occurring in the northeast populations are in conflict with the hypothesis of recent expansion towards the northeast of Brazil from Chaco populations. The alloenzyme data can also be interpreted as an ancient fragmentation among the Brazilian populations of *D. buzzatii*, allowing a differentiation and the non-assortment of the polymorphic chromosome inversions (Barker et al., 1985; Figueiredo & Sene, 1992). This is more significant if one considers that recently colonized areas in Spain and Australia, in which a significant population bottleneck occurred, show high chromosomal polymorphism (Knibb & Barker, 1988; Hasson, Naveira & Fontdevila, 1992). Estimates, using molecular data, show that an expansion of population size of the southeast of Brazil occurred at least 100,000 years ago indicating a pre-Holocene presence of *D. buzzatii* in Brazil which predates human colonization (DeBrito, Manfrin & Sene, 2002b).

The results mentioned here do not support the hypothesis of passive dispersion of *D. buzzatii* throughout Brazil and also do not support a natural dispersion following fluctuations in the distribution of the cacti during the Quaternary Period. The high level of polymorphism found in *D. buzzatii* populations in the northeast of Brazil, as well as the presence of private alleles in this region (Barker et al., 1985), suggest that *D. buzzatii* is distributed throughout Argentina and Brazil and that the concept of a center of origin in Chaco domain must be revised.

## Systematics

The first systematics analysis, and upon which many hypotheses about the history of the *D. buzzatii* cluster were developed, was based on data from paracentric chromosomal inversions (Ruiz, Fontdevila & Wasserman, 1982; Wasserman & Richardson, 1987; Tosi & Sene, 1989; Ruiz & Wasserman, 1993). These data suggest the monophyly of the cluster due to the fact that their species share the *Xabc* 2abmnz$^{7}$ 3b arrangement (Ruiz & Wasserman, 1993) that includes the addition of the 2 mnz$^{7}$ inversions to the *repleta* group primitive I arrangement (Figure 3). However, the intra cluster relationships cannot be defined on the basis of chromosomal inversions because there are few informative inversions within the cluster and each species has its own polymorphic inversions. The chromosome 2 of the *D. buzzatii* species is the standard for the *D. buzzatii* cluster (Ruiz & Wasserman, 1993). Each one of the other six species has, in addition to this standard, a fixed inversion in chromosome 2: *D. koepferae* has the 2j$^{9}$ inversion; *D. serido* and *D. antonietae* have the 2x$^{7}$ inversion; and *D. borbor-*

*ema*, *D. seriema* and *D. gouveai* share the $2e^8$ inversion (Tosi & Sene, 1989; Ruiz & Wasserman, 1993; Ruiz et al., 2000) (Figures 3 and 4). When only chromosome 2 is analyzed, the *D. buzzatii* species is the least derived within the group, and is at the base of the branching of the species of the cluster (Figures 3 and 4). In relation to the phylogeny, only the fixed inversion $2x^7$ shared by *D. serido* and *D. antonietae* species, and the $2e^8$ fixed inversion shared by *D. seriema*, *D. borborema* and *D. gouveai* species suggest a relationship between the *D. buzzatii* cluster species (Wasserman & Richardson, 1987; Tosi & Sene 1989; Kuhn et al., 1996; Ruiz & Wasserman, 1993; Ruiz et al., 2000) (Figure 4). For the intra-cluster relationship, the analysis based on similarity of the aedeagus morphology and patterns of chromosome inversions suggest a division of this cluster into two evolutionary lineages, the first formed exclusively by *D. buzzatii* species and the second one by the species of the *Drosophila serido* sibling set (Tidon-Sklorz & Sene, 2001) (Figure 3).

The first analysis using information from DNA sequences, aiming at developing a phylogenetic hypothesis for the *D. buzzatii* cluster, and its position within the *Drosophila buzzatii* complex, was performed with information from the mtDNA COI haplotypes (Manfrin, DeBrito & Sene, 2001). This analysis showed great similarities with the established relationships based on chromosomal inversions (Ruiz & Wasserman, 1993). The hypothesis established shows that the *D. buzzatii* cluster is a monophyletic group that, within the *D.buzzatii* complex, is a sister group of the *D. martensis* cluster. This result is consistent with data from inversions indicating a basal position of the *D. stalkeri* cluster within the *D. buzzatii* complex.

Based on mtDNA, inside the *D. buzzatii* cluster, the species are divided into two main branches suggesting two distinctive evolutionary lineages (Manfrin, DeBrito & Sene, 2001) (Figure 4). The most basal branch includes the individuals analyzed from the *Drosophila buzzatii* species. This information is similar to the morphological data (Tidon-Sklorz & Sene, 1995b; Moraes et al., 2004) and chromosomal inversions data (Ruiz & Wasserman, 1993), suggesting this one is the least derived species within the cluster. The *D. koepferae* species is allocated along with the *D. buzzatii* species, suggesting that this one is the sister species of *D. buzzatii*. Nevertheless, the information from different sets of data available for the cluster show that these species only share the tergite pattern and are lighter in color when compared to others species of cluster (Fontdevila et al., 1988). This information is not congruent with the aedeagus morphological analysis that includes *D. koepferae* inside the *Drosophila serido* sibling set (Silva & Sene, 1991). The second group defined by mtDNA COI haplotypes is comprised of all other species of the *D. buzzatii* cluster (Figure 4). Inside this evolutionary lineage, the species are divided into two well-supported clades. Individuals representing populations of *D. antonietae* comprise the first clade (Figure 4). The second clade includes populations of the *D. serido*, *D. gouveai*, *D. borborema* and *D. seriema* species. However, the phylogenetic relationships between these species were not totally resolved (Figure 4). Among these species, *D. antonietae* and *D. serido* share the fixed inversion $2x^7$, in disagreement with the molecular data analysis. The *D. gouveai*, *D. borborema* and *D. seriema* species share the $2e^8$ inversion; this information is consistent with the molecular data. *Drosophila seriema* is paraphyletic inside the cluster due to the great divergence observed among its populations and the data suggests that a haplotype of this species is the ancestor of the *D. borborema*, suggesting that these species are closely related. The haplotype of *D. serido* suggests this species is not monophyletic, and this information confirms data from karyotype and chromosomes polymorphic inversions that divide its populations into two groups: a group of population in the northeast of Brazil and another one along the coast (Baimai, Sene & Pereira, 1983; Tosi & Sene, 1989; Ruiz et al., 2000).

The analysis of the nuclear sequences which establishes the relationship among the *D. buzzatii* cluster species, considering all the cluster species until now, are restricted to the family of DNA satellites pBuM described for the *D. buzzatii* cluster (Kuhn et al., 1999; Kuhn et al., 2003; Kuhn & Sene, 2004, 2005). This family of satDNA can be divided into two subfamilies that are organized in separate chains. The first subfamily is denominated as pBuM−1 and has units of repetitions of 190 bp. The second subfamily is denominated as pBuM−2 and is formed by units of repetition that contain, besides the 190 bp of the pBuM-1 subfamily, a sequence of 180 bp, resulting in an unit of repetition of 370 bp. Sequences of

subfamily pBuM-1 are found in a great number of copies in *D. buzzatii*, *D. serido* and *D. antonietae*, but in a reduced number in the *D. borborema*, *D.gouveai* and *D. koepferae* species. Sequences of subfamily pBuM-2 are found in a great number of copies in *D. serido*, *D. antonietae*, *D. gouveai* and *D. seriema*, but in a small number of copies in *D. buzzatii*, *D. koepferae* and *D. borborema*. This information, added to the analysis of genetic distance, calculated for the sequence of the two subfamilies separately, divides the species of the cluster into three main branches (Figure 4). The first branching contains the *D. buzzatii* species; this information coincides with data from inversions, mtDNA and morphology that suggest that this species is the most basal species in the group (Ruiz & Wasserman, 1993; Tidon-Sklorz & Sene, 1995b; Manfrin, DeBrito & Sene, 2001; Moraes et al., 2004). The second branching contains *D. antonietae*; this information is partially similar to the data of mtDNA that also put this species in a separate branch from the other species. The third branching contains the *D. serido*, *D. gouveai*, *D. borborema*, *D. seriema* and *D. koepferae* species; this branch, with the exception of the *D. koepferae*, is totally coincidental with those produced by COI haplotype analysis (Manfrin, DeBrito & Sene, 2001).

### Secondary contact zone between *D. antonietae* and *D. serido* species

Analysis of populations in the coastal region of the states of Santa Catarina and Rio Grande do Sul identified an area of sympatry of *D. antonietae* and *D. serido* species characterized by polymorphic inversions (Ruiz et al., 2000). The aedeagus morphology analysis of individuals from these populations described an intermediate morphological form between types A (*D. serido*) and type D (*D. antonietae*) (Cansian & Sene, 2000). These data suggested that this region is a secondary contact area between the two species with hybrid formations. This contact area was limited to the north by populations of the Santa Catarina Island, on the coast of Santa Catarina state, where populations were identified as *D. serido*, and to the south by populations of Osório, in Rio Grande do Sul state, where populations were identified as *D. antonietae* (Silva & Sene, 1991). The data set results for these populations of allelic frequencies from allozymes (Morales, Manfrin & Sene, 2004) show that they are similar. However, among the individuals analyzed from the populations from Santa Catarina Island locality, the fast allele of IDH, characteristic of the *D. serido* populations from the northeast region of Brazil, was found always in homozygosis. The slow one that is characteristic of *D. antonietae* populations was found always in homozigosis also. The absence of heterozygotes for this allozyme and all previous data analyzed altogether reinforce the hypothesis of secondary contact between the *D. serido* and the *D. antonietae* species, suggesting a complex dynamic for this region. This secondary contact could have occurred due to the colonization of the south coast by the *D. serido* species finding *D. antonietae* which expanded its area of occurrence from the countryside of the state of Rio Grande do Sul throughout the coast towards the north (DeBrito, Manfrin & Sene, 2002a; Morales, Manfrin & Sene, 2004). With regard to the distinct patterns from the analyzed markers, some questions still remain: Were there events of hybridization and introgression? Are the species in sympatry? How many secondary contacts occurred? Have distinct events been added in order to determine the present pattern? When did they happen? When did this contact occur? In order to better understand the problem, we need a joint analysis of multiloci DNA sequence and information about the ecological basis of the population dynamics of these species.

### Ecological aspects

Specialization in relation to a host plant and its implications for the diversification of the *D. buzzatii* cluster is an important aspect, but there is little information about it. The biological model in the *D. buzzatii* cluster comprises the system *Drosophila*–yeast–cactus. This model involves diverse ecological conditions, such as chemical composition of the cactus, which can determine the spectrum of the yeast and bacteria that can develop during the decaying process of the cactus tissues (Starmer et al., 1990). Thus, the adaptive association of the *Drosophila* species to different species of cacti depends on the chemical composition of cactus and on different species of yeast (Kircher, 1982; Starmer, 1982).

Most information we have concerning the association *Drosophila*/host cacti was obtained from the observation of individuals that emerged from cacti decaying tissues collected in nature (Pereira, Vilela & Sene, 1983; Ruiz et al., 2000; Moraes & Sene, 2002). Few experiments were performed in order to obtain the measurements of viability and fecundity of flies in relation to different species of cacti.

The great amount of information about preference for oviposition sites and the influence of the host cacti to the fitness of individuals were attained for *D. buzzatii* and *D. koepferae* populations of the Chaco domain, where these species occur in sympatry (Fanara, Fontdevila & Hasson, 1999; Hasson, Naveira & Fontdevila, 1992; Fanara & Hasson, 2001). Amongst these species there is a certain degree of superposition of the niches; nevertheless, *D. buzzatii* explores cladodes of prickly pears cacti of the *Opuntia* genus, while *D. koepferae* is primarily associated with columnar cacti of the *Cereus* and *Trichocereus* genus and the viability in these species depends on the cactus species. These results, according to the authors, indicate that the evolution of the utilization of the host plant in this pair of species had entailed a host shift accompanied by changes in oviposition preference and oviposition behavior and other life history traits such as viability, developmental time and thorax length. Outside the Chaco Domain, the information available in the literature shows that the *D. buzzatii* species prefer species of *Opuntia* genus for oviposition, but frequently emerges from columnar cacti of different genus (Pereira, Vilela & Sene, 1983; Ruiz et al., 2000).

The *Drosophila antonietae* is mainly associated with the cactus *Cereus hildmaniannus*. With respect to this association, we cannot say that it is the result of adaptation, since in areas of occurrence of *D. antonietae* it is the only species of columnar cacti observed. Nevertheless, adults from this species emerge from decaying tissues of *Opuntia monacantha*, where this cactus occurs in sympatry with *Cereus hildmaniannus* (Ruiz et al., 2000; Tidon-Sklorz & Sene, 2001).

The populations of *Drosophila gouveai* are associated with *Pilosocereus machrisis* at the extreme southernmost of its distribution, in regions where this is the only species of cactus found (Moraes & Sene, 2002). In other regions of *D. gouveai* distribution, a variety of cacti genus are observed in sympatry and it has not been possible to identify an association between the *Drosophila* and the cactus (Pereira, Vilela & Sene, 1983; Tidon-Sklorz & Sene, 2001).

*Drosophila borborema*, *D. serido* and *D. seriema* populations from the northeast of Brazil occur in sympatry in many regions in which the diversity and density of cacti is high (Vilela & Sene, 1977; Vilela, Pereira & Sene, 1983; Tidon-Sklorz & Sene, 1995a). Thus, for these species there is no information about the association cacti/*Drosophila*. Only one record exists for *D. borborema* in which adults emerged from the *Pilosocereus piauhyensis* (Vilela & Sene, 1977). In populations of *D. serido* from the Atlantic coast, the host cactus is *Cereus pernanbucensis*, which occurs in the southern region of the state of São Paulo, followed by the *Opuntia monacantha* cactus, which occurs up to the coast region of the state of Santa Catarina (Vilela & Sene, 1977; Bizzo & Sene, 1982; Ruiz et al., 2000).

## Evolutionary history – concluding remarks

The *Drosophila buzzatii* cluster is composed of seven cactophilic species and it has been studied, within a context of group of species, since the 1970s (Vilela & Sene, 1977; Sene, Pereira & Vilela, 1988). It is a biological model with the following characteristics: (1) it is a monophyletic group, which is fundamental for evolutionary comparative studies, such as the study of evolution of characters; (2) it has a great quantity of genetic and ecological information, all associated to geographic distribution; (3) it has significant systematic information; (4) it has a relatively low taxonomic diversity, which allows for studies on intra- and inter-specific levels for hypothetical tests; (5) it has ecological restrictions, that allow the location and delimitation of populations in natural environments.

The species of the *D. buzzatii* cluster, as with most of the *repleta* group, are sibling species and the aedeagus is the main diagnostic character for taxonomic analysis (Vilela, 1983). Nevertheless, unlike the *repleta* group in which a great interspecific diversity exists in this structure, in the *D. buzzatii* cluster, the differences between the species (especially from the *Drosophila serido* sibling set) are quantitative. As shown by Silva and

Sene (1991), some regions of the aedeagus are more informative than others; these regions are the arches, delimited by points of homology, from the superior distal region (Figure 2). In a pairwise comparative analysis, using measurements of curvature, it was observed that the curvature and length of the arch III are discriminated among the *D. gouveai* and *D. antonietae* species. (Costa & Cesar, 2000; Prado et al., 2004). Franco et al. (2004) showed that the measurements for arch IV, which define the distal inferior portion of extremity of the aedeagus, are the ones that discriminate the *D. antonietae* and *D. serido* species. These results suggest that, in the species of *D. buzzatii* cluster, the distal portion (tip) of the aedeagus (arch III and IV) that shows the largest inter-specific differences, can be considered a hot evolutionary spot (Kulikov et al., 2004), independent from other parts of this structure. This would be an important aspect for future analysis of genetic architecture of traits involved in the cladogenesis in the *D. buzzatii* cluster.

A comparative analysis of the wing morphology was performed among the species of the *D. buzzatii* cluster, in order to verify the possibility of using this structure as a diagnostic character for the identification of these species. The results showed that the morphology of the wing in the *D. buzzatii* cluster is an important taxonomic character, separating 98% of the individuals according to the species (Moraes et al., 2004). The position of the latter cross-vein and costal vein, as well as its size, is the most important character for discrimination in this cluster. An analysis of phenetic distance of these data clustered all species, except *D. buzzatii* (indicating that it was the most divergent species in the cluster) (Figure 4). Within the main branch, *D. seriema* and *D. koepferae* were isolated from the branch clustering the remaining species. *D. antonietae* and *D. gouveai* were clustered in the same branch, whereas *D. borborema* and *D. serido* were clustered in another one.

All the studies involving the *D. buzzati* cluster species, always considering the species geographical distribution, have shown that the distribution of variability, either chromosomal, or morphological and molecular, is frequently fragmented with congruence between the markers. This pattern of distribution suggests vicariant events in the differentiation of the populations and species of the *D. buzzatii* cluster (Silva & Sene, 1991; Sene, Pereira & Vilela, 1982, 1988).

The analysis of the geographic distribution of the haplotypes of mtCOI found for the species in the cluster shows a pattern of divergence that, according to Avise (1989), suggests differentiation in geographically isolated taxa. Three mitochondrial groups were observed in the *D. buzzatii* cluster (Manfrin, DeBrito & Sene, 2001). One group can be considered as characteristic of the Chaco domain, including *D. koepferae* and *D. buzzatii*, which is basal in the *D. buzzatii* cluster. The second one occurs in the South/Southeastern region of South America, basically in the Paraná and Paraguay River basins, which includes the populations of the *D. antonietae* species. The third mitochondrial group includes the species *D. serido*, *D. seriema*, *D. gouveai* and *D. borborema* and occurs in the northeast of Brazil. This geographic discontinuity observed could be evidence of geographical barriers preventing gene flow and allowing the accumulation of genetic differences between the populations. This discontinuity is also observed when the distribution of the fixed and polymorphic inversions is analyzed for the species from the *D. buzzatii* cluster (Tosi & Sene, 1989; Ruiz et al., 2000).

The *D. buzzatii* cluster species geographic distribution and the discontinuity variation observed in the markers coincide with areas of endemism defined for other groups of insects (Amorim & Pires, 1996) and plants (Oliveira & Ratter, 1995). This congruence between distribution and geographic breaks among groups of animals and plants from independent phyletic origin suggest events of vicariance (past fragmentation), due to geographical barriers, in the differentiation of the species. For the *D. buzzatii* cluster, these barriers would have determined the processes of speciation and defined the nuclei areas of distribution for each species. The comparison with the geological time estimated by Amorim and Pires (1996), suggest that the breaks in the distribution and isolation of the groups that compose the *D buzzatii* cluster occurred approximately in the middle of the Tertiary Period. Considering the substitution rates of nucleotides of $6.85\times10^{-9}$ per site per year for the mtDNA, the estimated age for the separation of the clades containing *D. koepferae* and *D. buzzatii* of the clade containing the other species of the *D. buzzatii* cluster is 6–12 Myr, while the separation of the clade comprising the *D. antonietae* species and the clade comprising the

species *D. serido*, *D. seriema*, *D. gouveai* and *D. borborema* is estimated at 3–6 Myr.

For the *D. buzzatii* cluster differentiation processes, the pattern of distribution of the variation for the different markers and the ages of divergence proposed suggests pre-Quaternary vicariant events. This pattern contradicts the hypothesis which proposes that all species of the cluster, except for *D. buzzatii*, differentiated themselves more or less simultaneously in a mosaic fashion from the isolation of populations, with a continuous geographic distribution, during climatic paleocycles of the Quaternary (Sene, Pereira & Vilela, 1982, 1988; Tosi & Sene, 1989).

In spite of the discontinuity and association of endemic areas defined for South America, some populations, especially those on the limits of distribution of the species, present conflicting information indicating secondary contact with some level of hybridization, introgression or sympatry. This secondary contact could have occurred as a consequence of expansion of the area of occurrence for these species during paleo climatic alterations, as those promoted by the glacial cycles of the Quaternary. Paleoevents of climatic changes, such as glacial cycles, that occurred since the end of the Pliocene, theoretically changed the distribution of the vegetation on the American Continent (Bigarella, Andrade-Lima & Riehs, 1975; Ab'Saber, 1977) allowing the range expansion of cacti populations in periods of dry and cold climates and their retraction in humid and hot climates. It is very likely that these cactophilic *Drosophila* species have also suffered expansions and contractions of its distribution, which is consistent with the current observation of secondary contact areas (Silva & Sene, 1991; Ruiz et al., 2000; Manfrin, DeBrito & Sene, 2001; DeBrito, Manfrin & Sene, 2002a). The expansion of geographic areas is the most frequent and significant event in a phylogeographic analysis performed for the *D. buzzatii* cluster (DeBrito, Manfrin & Sene, 2002a, b). Altogether, this information indicates that part of the distribution of the species and the populations structure detected in the *D. buzzatii* cluster is the result of events of past fragmentation and expansion of geographic areas due to the paleoecology events in South America, which are historical events rather than recurrent forces.

Some works have suggested that the population structure of some species of the *D. buzzatii* cluster can be defined by the occurrence of gene flow, causing little population structure, or due to geographic isolation, suggesting isolated populations in models of islands (Moraes & Sene, 2002; Machado et al., 2003; Mateus et al., 2003). These population studies suggest that recurrent events determine the distribution of the variation between the populations of the *D. buzzatii* cluster. Today, the distribution of the species and the population variation is the result of historical and recurrent events overlapping throughout the evolutionary process of the cluster. The consequences and influences of each of these events are related to the distribution, size and ecological aspects of each population and species of the *D. buzzatii* cluster. According to Futuyma (1998), "whether one is studying the genetic structure of populations, the adaptations of species, or the structure of ecological communities, it is important to bear in mind that history, especially recent dynamics such as those of the Pleistocene, casts its shadow on the present. The vicissitudes of the Pleistocene must have destabilized many genetic and ecological equilibrium, and the glaciers had hardly retreated when major new disruptions began".

In the analysis of the information for the *D. buzzatii* cluster, as for any other group, we must also consider the nature of the marker which is being analyzed and that each characteristic of a group of individuals are the result of specific loci forces and different types of inheritances. For the *D. buzzatii* cluster species, although there is a general agreement of the information from the markers used, some contrasting patterns of differentiation still remain, as shown by Sene, Pereira and Vilela (1988), using only classical genetic markers. The mtDNA information is the most contrasting one: (1) it is the only marker that allocated *D. koepferae* as the closest one to *D. buzzatii* rather than the other species from *Drosophila serido* sibling set; (2) it is the only marker that classified some populations from *D. gouveai*, further South from its distribution, as being *D. antonietae*; (3) it is the only marker that, with haplotypes that have a number of nucleotide differences equivalent to the one found amongst the other species of the *D. buzzatii* cluster, differentiates the populations of *D. seriema*. All these cases have in common the sympatry among populations of different species suggesting a process of secondary contact, since it is assumed that the

differentiation between the species from the *D. buzzatii* cluster occurred in allopatry. The hypothesis to explain the disagreement between the information obtained from various markers and the information of the mtDNA is based on the occurrence of introgressive hybridization of cytoplasmatic material among the populations under secondary contacts. In case of hybridization, mtDNA tends to introgress faster than nuclear markers, characterizing the distinct behaviors of mtDNA and nuclear markers that are being observed in hybrid zones, the so-called cytonuclear disequilibrium (Arnold, 1993).

In an event of introgressive hybridization, an eventual break of genomic equilibrium of the hybrids can occur, and natural selection tends to eliminate, in each parental species, the foreign genetic material (Carson, 1987; Templeton, 1989). This would explain the fact that only foreign cytoplasmatic DNA, which had passed by the event of introgression, remains in the populations. Since the mtDNA is one of the most used markers for phylogenetic analysis, caution must be taken when phylogenies hypothesis are proposed with using only this marker.

The *D. buzzatii* cluster populations' structure shows that even closely related species, submitted to the same environment, react differently in an evolutionary sense. This is evident when one compares the distribution of the variation between populations of the *D. buzzatii* species and other populations of the *D. serido* sibling set. *D. buzzatii* has a large geographic distribution, and is sympatric with practically all species of the *Drosophila serido* sibling set. Nevertheless, among *D. buzzatii* populations there is no significant differentiation considering the markers used, except for chromosome inversion polymorphism in the Chaco domain. If the present geographic distribution of the *D. buzzatii* is as ancient as the species of the *Drosophila serido* sibling set, as suggested by various markers, it also must have suffered the events of historical fragmentation, suggested by the *Drosophila serido* sibling set, reacting in a different way to the events. This classifies the *D. buzzatii* as a species with high genomic cohesion, in agreement with the hypothesis elaborated by Carson and Wasserman (1965) and later by other researchers. *Drosophila buzzatii*, besides being a cactophilic species that does not have a specific host, is easily maintained in the laboratory even in the absence of cactus, and shows a great capacity for survival when introduced, in a passive way, with its hosts. It is the only cactophilic species that became semi-cosmopolitan, found today in Africa, Europe, Asia and Australia.

For the *D. serido* sibling set the situation of differentiation is completely different. The populations are differentiated in six species and inside of them there are polytypic populations. This suggests that the species of *Drosophila serido* sibling set has a larger potential for differentiation.

As a hypothesis to be tested, the non-definition of relationship in the *D. buzzatii* cluster, as well as the conflicting information from different markers, could be the result of adaptive radiation of the cluster. The same is suggested for the *Drosophila repleta* group where all its subgroups derived directly from the chromosomal arrangement Primitive I including the *D. buzzatii* species complex (Wasserman, 1992; Ruiz & Wasserman, 1993; Diniz & Sene, 2004) and combined data analysis of different genes generate phylogenies poorly supported on the base of the tree (Durando et al., 2000). An analysis of DNA multi-loci sequences will be the next step in an attempt to define the relationships between the species of the *D. buzzatii* cluster, in order to add more information about its evolutionary history.

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