Table 1. Frequencies of chromosomal arrangements for wild males and sons of wild females (Avala Mountain population). The number of individuals is indicated by *n.*

Chromosome arrangements	Wild males		Sons of wild females	
	n	%	*n*	%
A_{st}	7	31.8	13	46.4
A_1	10	45.5	8	28.6
A_2	5	22.7	7	25.0
Total	22		28	
J_{st}	7	15.9	15	26.8
J_1	37	84.1	41	73.2
Total	44		56	
U_{st}	2	4.5	6	10.7
$U_{\underline{1}+\underline{2}}$	24	54.6	33	58.9
$U_{\underline{1+2+6}}$	13	29.5	12	21.4
$U_{\underline{1+8}+\underline{2}}$	5	11.4	5	8.9
Total	44		56	
E_{st}	7	15.9	15	26.8
$E_{\underline{1+2}}$	1	2.3	1	1.8
$E_{\underline{1+2+9}}$	20	45.5	25	44.6
$E_{\underline{1+2+9+12}}$	3	6.8	1	1.8
E_8	13	29.5	14	25.0
Total	44		56	
O_{st}	6	13.6	12	21.4
$O_{\underline{3+4}}$	18	40.9	26	46.4
$O_{\underline{3+4}+\underline{1}}$	3	6.8	8	14.3
$O_{\underline{3+4}+\underline{2}}$	2	4.5	/	/
$O_{\underline{3+4}+\underline{5}}$	1	2.3	1	1.8
$O_{\underline{3+4}+\underline{6}}$	1	2.3	1	1.8
$O_{\underline{3+4}+\underline{7}}$	1	2.3	/	/
$O_{\underline{3+4+8}}$	5	11.4	2	3.6
$O_{\underline{3+4}+\underline{17}}$	1	2.3	/	/
$O_{\underline{3+4}+\underline{22}}$	6	13.6	6	10.7
Total	44		56	

mixtum at 450 m a.s.l. (Zivanovic and Mestres, 2010). Males and sons of wild females were crossed with virgin females of the Küsnacht strain. Third instar larvae from F_1 were dissected to obtain the salivary glands and the polytene chromosomes were stained and squashed in aceto-orcein solution. In Table 1, we present the chromosomal polymorphism obtained for both groups (males and sons of wild females).

Fisher's exact test was used to compare the chromosomal composition of wild males and sons of wild females (statistically significant *p-value* < 0.05). This test is more precise than chi-squared when the expected frequencies are small (Zivanovic *et al.*, 2014). The corresponding *p-values* were obtained by bootstrap procedure (100000 runs). No significant differences were observed for any chromosome of the karyotype: A (*p-value* = 0.485), J (*p-value* = 0.230), U (*p-value* =0.572), E (*p-value* = 0.536), and O (*p-value* = 0.338). Thus, it seems that the two groups can be grouped together to obtain the chromosomal polymorphism of the population.

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Monthly fluctuations in abundance of *Drosophila willistoni* and the relationship with rainfall in the northern region of the Brazilian Atlantic Forest.

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Introduction

The Atlantic Forest comprises communities of trees that reach 35 m in height, which are surrounded by treelets, bushes, and a variety of epiphytical species (Klein, 1978). The biome stretches over a large region, between latitude 4ºS and 32ºS, along a wide array of climatic zones and vegetation physiognomies, from tropical to subtropical (Tabarelli *et al*., 2005).

The Atlantic Forest is subject to a diversity of abiotic conditions that promote endemisms and high species diversity (Campanili and Schaffer, 2010). However, such diversity is exposed to severe conservation issues. The Atlantic Forest stretches along the 4,000-km Brazilian coastline, which is the country's area with the highest population density and where most economic activities take place (Campanili and Prochnow, 2006). These factors are responsible for the historical destruction of the biome. Today, there remains less than 9% of the original cover of the Atlantic Forest (Fundação SOS Mata Atlântica, 2013).

The Neotropical regions, mainly in their forested areas, are home to approximately one third of the world's insect species. These organisms account for over 50% of the genes, of biomass volumes, and of energy transfer phenomena in terrestrial ecosystems. Therefore, conservation of insect populations plays an essential role in the preservation of these habitats (Brown, 1989). More specifically in the Atlantic Forest, insects stand out compared to other organisms due to their high species richness and considerable abundance of individuals. In this sense, drosophilids have become the object of an increasing number of ecological studies (De Toni *et al*., 2007; Cavasini *et al*., 2008; Döge *et al*., 2008; Garcia *et al*., 2008; Mata *et al*., 2008; Gottschalk *et al*., 2009; Bizzo *et al*., 2010; Rohde *et al*., 2010; Garcia *et al*., 2012). Such preference is explained mainly in light of the fact that these flies are extremely sensitive to environmental changes. However, drosophilids make an interesting model also because of practical advantages, which include high population numbers, small size of individuals, short lifecycle, and low capture effort (Powell, 1997; Mata *et al*., 2008; Garcia *et al*., 2014).

Drosophila willistoni is one of the most abundant drosophilids in the Brazilian Atlantic Forest. In spite of that, the species has been but poorly investigated in the northernmost parts of the biome (Gottschalk *et al*., 2008). It belongs to the *willistoni* group, which comprises 23 species and is widely distributed, from the south of the United States down to Uruguay and northern Argentina (Spassky *et al*., 1971; Ehrman and Powell, 1982). Despite this abundance, most ecological studies have not properly addressed the identification of these individuals to species level, since they belong to a group of cryptic species (the *willistoni* subgroup).

The present study investigates the monthly fluctuations in abundance of *D. willistoni* along one year and assesses the relationship of this species' abundance with rainfall in the northern region of the Atlantic Forest.

Materials and Methods

Drosophilids were collected once a month for a year, starting in May 2012, in Dois Irmãos Park (8°0'25.9"S, 34°56'49.1"W), municipality of Recife, state of Pernambuco (PE), Brazil. The area is a conservation unit under the responsibility of the state of PE. It covers approximately 370 ha, where altitude varies between 30 and 80 m a.s.l, and topography is undulated (Machado *et al*., 1998). Vegetation cover is classified as dense ombrophilous forest. Normally, the rainy season starts in March and ends in August, and mean yearly rainfall is 2,400 mm (Andrade-Lima, 1961). The area has been completely enclosed by the city of Recife in its growth process, becoming an "island" of Atlantic Forest inside an urban center (Figure 1).

Drosophilids were captured using the trap described by Tidon and Sene (1988). In each collection, 10 traps containing banana baits were hung from trees 1.5 m above the ground level. Traps were spaced 30 m away from one another along the transect, and were left in place for three consecutive days.

The individuals that belonged to the *willistoni* subgroup were initially sorted from the remaining flies based on external morphology characteristics. The representatives of *D. willistoni* were identified to species level with the analysis of male genitalia (Wheeler and Kambysellis, 1966), according to Malogolowkin (1952) and Rohde *et al*. (2010). Females of the *willistoni* subgroup were differentially classified to species level based on the number of males identified.

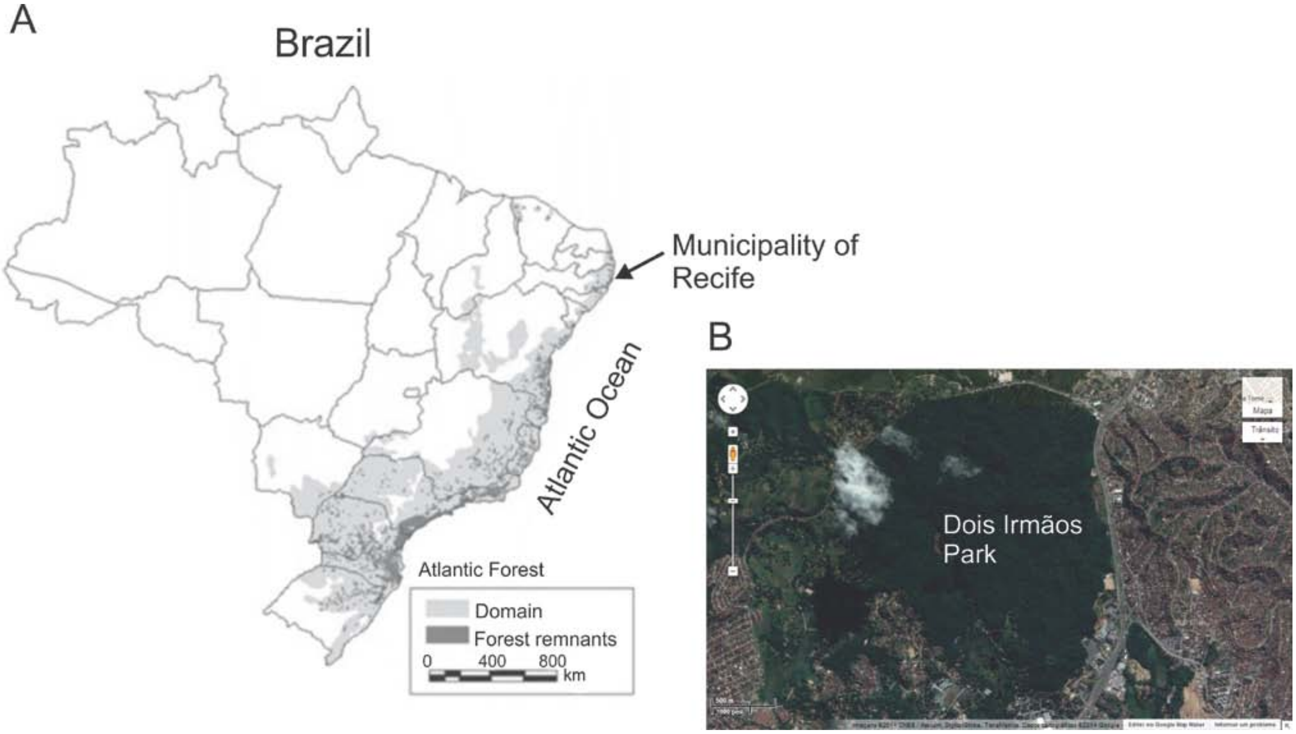

Figure 1. (A) Map of Brazil showing the municipality of Recife and the distribution of the Atlantic Forest biome domain and remnants, according to Fundação SOS Mata Atlântica and Instituto Nacional de Pesquisas Espaciais (2010). (B) Satellite image showing Dois Irmãos Park, where drosophilids were collected, and part of the city of Recife. Source: Google Earth (2014).

Along the collection period, rainfall data in the study site were obtained from the local climate and hydrological resources agency (APAC, 2014). The relationship between abundance of *D. willistoni* and rainfall was evaluated using the Pearson Correlation criterion (r). Qualitative correlation was assessed using the criteria described by Callegari-Jacques (2003), where r values are 0 (no correlation), 0.01 - 0.3 (weak), 0.31 - 0.6 (moderate), 0.61 - 0.9 (strong), 0.91 - 0.99 (very strong), and r = 1 (full correlation). The test was carried out using the Biostat software (Ayres *et al*., 2007).

Results and Discussion

In total, 15,301 drosophilids were collected, of which 13.70% were identified as *D. willistoni*. The lowest relative abundance of the species was recorded in January 2013 (4.66%). In July, September and October, abundance values accounted for over 30% of the individuals collected. The highest number of individuals of the species was captured in July (44.05%). This peak in abundance virtually coincided with the maximum rainfall value (Figure 2).

Significant and strong correlation was observed between absolute abundance of *D. willistoni* and rainfall ($r = 0.66$; $p = 0.02$).

Studies on the ecology of drosophilids that differentiate the representatives of the *willistoni* subgroup to species levels are scarce (Garcia *et al*., 2014). Still rarer are those that aim to shed more light on the seasonal fluctuations in the numbers of these flies along periods of up to one year. Research has more consistently addressed the abundance of *D. willistoni* in the southern parts of the Atlantic Forest (De Toni and Hofmann, 1995; Garcia *et al*., 2008; Garcia *et al*., 2012). These studies, as observed in the present investigation, for the northern region of the Atlantic Forest, reported that the abundance of *D. willistoni* was greater in periods with higher rainfall.

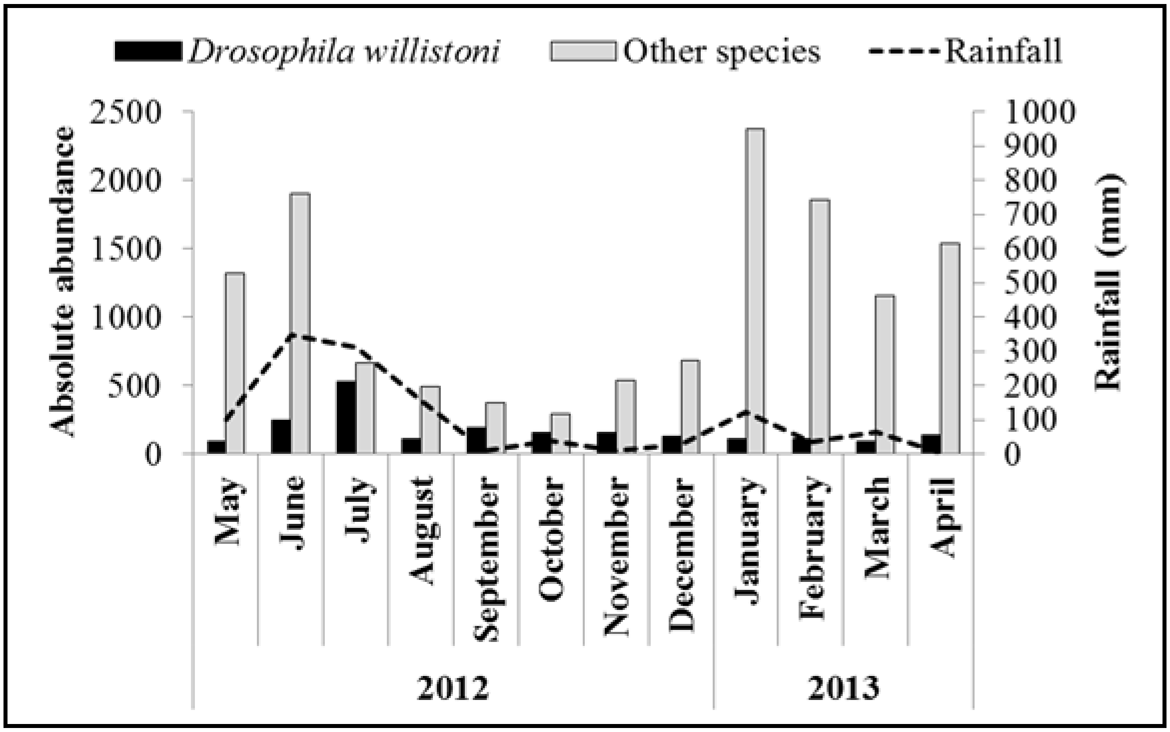

Figure 2. Oscillation in absolute abundance of *Drosophila willistoni* and rainfall along a 12-month period in Dois Irmãos Park, Recife, PE, Brazil.

Garcia *et al*. (2014) investigated the representativeness of *D. willistoni* in periods of varying rainfall in the northern region of the Atlantic Forest in Brazil. The authors detected that the greatest abundance of the species was during the rainy season. The same pattern was observed in the Caatinga biome. Other studies reported similar findings for the Cerrado biome (Valadão *et al*., 2010; Roque *et al*., 2013), but not for the Pampa biome (Poppe *et al*., 2013).

The present study contributes with new information about the representativeness and monthly fluctuations of *D. willistoni* in the northern part of the Atlantic Forest. Since species respond differently to abiotic factors, knowing how they affect populations is essential in order to understand interspecies interactions.

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Lack of evidence for directional selection on *Sex combs reduced* gene in *Drosophila* species differing in sex comb morphology.

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Introduction

Sex combs are secondary sex characteristics located on the first and second tarsals segments in males of some *Drosophila* species. They are characterized by a set of one or two modulated transverse bristle rows which point distally, and are oriented along the proximo-distal axis of the prothoracic legs. Sex combs can influence male mating success (*e.g.*, Ng and Kopp, 2008). While sex combs may have originally evolved for pushing female wings apart prior to copulation, the combs are now used for varying purposes across *Drosophila* subspecies (Spieth, 1952). For example, in the *obscura* group, sex combs aid in the abdominal attachment of the male to the female. When sex combs have been reduced, copulation rates have consequently dropped (Spieth, 1952).

Dramatic phenotypic variations exist in sex comb morphology between species of *Drosophila*, but the genetic underpinnings of this variation are still poorly understood (Graze *et al.*, 2007). In this study, we focus on three species in the *obscura* group of *Drosophila* differing in the number of sex comb rows per foreleg and number of teeth per comb. Figure 1 illustrates the variation in sex combs of the species examined here. *D. subobscura* males have large sex combs on both the first and second tarsal segments, with the combs lying perpendicular to the tarsus (Beckenbach and Prevosti, 1986). *D. pseudoobscura* have smaller sex combs on both segments than *D. subobscura* (Beckenbach and Prevosti, 1986). Finally, *D. azteca* has a still smaller sex