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Structure of Drosophilidae Assemblage (Insecta, Diptera) in Pampa Biome (São Luiz Gonzaga, RS)

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ABSTRACT

The Brazilian Pampa (the southernmost end of the country) is currently a highly modified environment because of increasing agricultural activities. In many places, only small parts of grasslands remain inside an agricultural landscape. Drosophilidae (Diptera) have been widely used as a potential bioindicators to monitor the effects of anthropogenic changes in natural environments. However, the fauna of Drosophilidae in the Pampa Biome from natural and disturbed environments, still remains largely unknown. The present study represents one of the first attempts to fill this gap, showing results from monthly collections in the municipality of São Luiz Gonzaga (28°24'28"S, 54°57'39"W), in the Brazilian Pampa. A species inventory was carried out in two contrasting environments, an urban zone and a forest remnant (rural zone). In both areas banana-baited traps were used to capture adult drosophilids. The identification was made using external morphology and male terminalia. In total, 13,379 drosophilids were analyzed (rural zone: N *= 8,812 and* S_{obs} *= 25; urban zone:* N *= 4,567 and* S_{obs} *= 16). In the present study, 16 (60%) out of 26 species were found exclusively or preferentially in the forest. The period of highest richness was between the months of June to November (roughly winter and spring), and the period of lowest richness was from December to May (roughly summer and autumn). An analysis of cluster by the Coefficient of Jaccard showed that species composition slightly changes when the period of the year with higher temperatures (from January to May) is compared with the period with lower temperatures (from June to October). The species abundances were also highly affected by seasonality, as revealed by the Morisita Index, since the samples clustered into similar groups in consecutive periods and in the same season, showing the seasonal preference of some species. The time component was a determinant in the diversity of the assemblage, surpassing the spatial effect. The strong reduction in diversity in the urban area when compared to a small forest patch is evidence of the importance of the natural environments in maintaining the diversity in the Pampa biome, currently a highly disturbed landscape.*

Key-Words: Pampa Biome; Drosophilidae; Diversity; Bioindicator.

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INTRODUCTION

The loss of biodiversity has become a central issue, with the recognition that the increasing human pressure on landscapes and natural habitats results in population or species extinction at unprecedented rates. Populations are entities in a continuous process of change. Even when the community and the ecosystem do not seem to be changing, intrinsic factors like the density, mortality, birth rate, food availability, among others, are in constant fluctuation, and the species that compose this community keep in constant adjustment for these changes (Odum, 1988). The environment change affects the occurrence and the abundance of some resources used by species that in turn may respond differently to these alterations. In this sense, it is possible to distinguish two kinds of time-based change: the predictable change (like the daily variation and the seasonal variation), which can increase or decrease the diversity of local species, and the unpredictable change (stochastic events, and catastrophic sometimes), which can cause a decrease in specific diversity (Begon *et al.,* 1996). Changes in land use, including urbanization and agricultural expansion, concomitant with the fragmentation and disturbance of the natural environments, may substantially alter species distributions and diversity. The existence of large gaps in species occurrence data and comprehensive monitoring schemes are, therefore, strong impediments to the detection of these processes (Kivinen, 2007).

The Brazilian Pampa is currently a highly modified environment. The Pampa Biome is a landscape mostly neglected by biodiversity studies, despite its high diversity and characteristic wildlife and flora. This ecosystem extends over an area of approximately 700,000 km^2 of mainly plain lowlands, shared between Argentina, Brazil and Uruguay (Bilenca & Miñarro, 2004). In Brazil, it covers the southernmost end of the country, in the state of Rio Grande do Sul. This portion represents about 176,000 km^2, approximately 63% of the area of the state and 2.1% of the Brazilian territory (Collares, 2006). The original landscape is predominantly covered by grasslands, although these are sometimes naturally invaded by arboreal formations of deciduous seasonal forest and ombrophilous dense forest, remarkably in northern and eastern parts of the State of Rio Grande do Sul (IBGE, 2004), where the biome is bordered by the Atlantic Forest biome. Unfortunately, the Pampa has been suffering a wide loss of diversity and habitat due to the fast agricultural expansion started in the 1970's, aggravated recently by plans to convert wide areas of grasslands to monoculture of trees, according to the Agricultural Census (IBGE, 2006). In many places, only small parts of grasslands remain inside an agricultural landscape (Risser, 1997; Porto, 2002; Bencke, 2003). Currently, just 11.7% of the Pampa Biome has been spared human influence in Rio Grande do Sul (PROBIO, 2007).

Flies of family Drosophilidae (Diptera) have been widely used in scientific research as a paradigmatic model and more recently have become a target taxon of biodiversity inventories and suggested as a potential bioindicator to monitor the effects of anthropogenic changes in natural environments (Avondet *et al.,* 2003; Ferreira & Tidon, 2005; Gottschalk *et al.,* 2007). They are a diverse and relatively well-known taxon, easily sampled with a low cost, and very sensitive to environmental changes. However, the fauna of Drosophilidae in the Pampa Biome, both from natural and disturbed environments, still remains largely unknown. Although the state of Rio Grande do Sul has been one of the most targeted study areas in Brazil concerning Drosophilidae diversity, most of the studies have been conducted in localities belonging to the Atlantic Forest Biome (Petersen, 1960; Franck & Valente, 1985; and others), while the Pampa Biome has been largely neglected, being one of the most unexplored in Brazil, as noted by Gottschalk *et al.* (2008). In fact, the only Drosophilidae diversity inventories performed in this Biome in Brazil sampled localities in or nearby the city of Porto Alegre: a forested area at Itapuã State Park (Valente & Araújo, 1991), a rural grassland area in Guaíba (Saavedra *et al.,* 1995) and the urban region of the city of Porto Alegre (Silva *et al.,* 2005; Garcia *et al.,* 2008; Garcia *et al.,* 2012). Recently, Hochmüller *et al.* (2010) conducted a survey in a transition area between Pampa Biome and Atlantic Forest Biome in the municipality of Cruz Alta. Similarly, outside Brazil, only a few Drosophilidae inventories have been conducted in the biome, a study carried out in Argentina (Fernández Iriarte & Lopez, 1995) and one in Uruguay (Goñi *et al.,* 1997, 1998), besides sparse records.

The present study represents one of the first attempts to fill this gap, showing results from monthly collections in the municipality of São Luiz Gonzaga, Rio Grande do Sul, in the Brazilian Pampa. A biodiversity inventory was carried out in two contrasting environments, an urban zone and a forest remnant.

MATERIALS AND METHODS

Study area

The collections were carried out in two areas in the municipality of São Luiz Gonzaga (28°24'28"S,

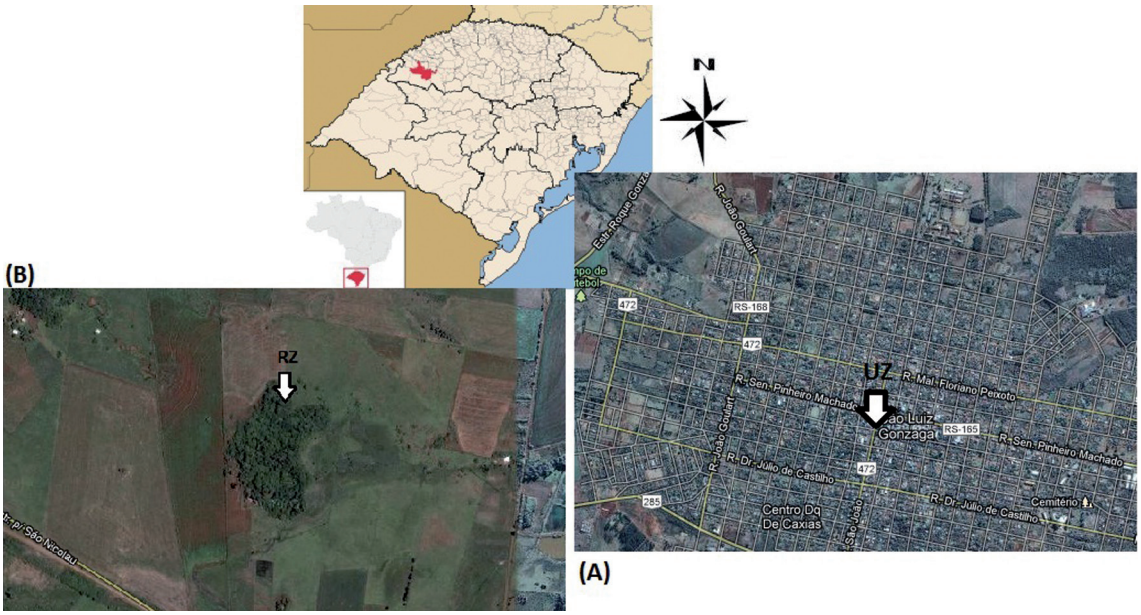

FIGURE 1: Rio Grande do Sul Map showing the municipality of São Luiz Gonzaga and the sampling zones: Urban Zone (A); Rural Zone (B). Source: http://maps.google.com.br/maps?hl=pt-BR&tab=wl&q=sao%20luiz%20gonzaga.

54°57'39"W), northwest of the state of Rio Grande do Sul, southern Brazil, a region of subtropical climate characterized by rainy weather and well defined seasons, with negative temperatures during the winter and a hot summer. The region has been heavily degraded, consisting nowadays of medium and small-sized cities in a predominantly agricultural landscape, with the natural grasslands highly disturbed and the forested areas reduced to just small patches of secondary forests. It is located near the northern border of the Pampa Biome, as defined by IBGE (2004).

Two contrasting localities were surveyed. The urban zone (UZ), in downtown (28°24'390"S, 54°57'371"W), is situated in the main urban and commercial area of the municipality (Fig. 1a). According to the criteria described by Ruszczyk (1986/1987), based on percentage of vegetal cover like was done by Gottschalk *et al.* (2007) in Florianópolis, this area can be considered as having a medium urbanization level. The rural zone (RZ), located about 10 km from downtown collection point (28°22'51.2"S, 55°00'8.62"W), is a small native fragment of deciduous seasonal forest inside a region originally with predominance of steppe savanna, today largely replaced with agricultural areas (Fig. 1b).

Collections and identification

In both areas banana-baited traps (Tidon & Sene, 1988) were used to capture adult drosophilids. For each sample, one kilogram of banana were mashed, sprinkled with baker's yeast and distributed in 5 traps hung in the trees at about 1.5 m above the ground, where they were kept for five days. Samples were taken monthly from September 2007 to September 2008, and in November 2008 and January 2009.

Flies were maintained in ethanol 70% until identification. The identification was made using external morphology and male terminalia, consulting specialized literature. Analysis of male terminalia was conducted according to Bächli *et al.* (2004).

Some individuals belonging to *Drosophila repleta, D. tripunctata* and *D. guarani* species groups that remained unidentified at species level were not scored for statistical analysis of species abundance and diversity measures (just ~ 8% of total sample). However, they were considered in the total number of individuals (N) and the number of individuals of Neotropical species (N_{nat}).

Voucher specimens of the material collected were deposited in the Laboratory of Zoology of Universidade Regional Integrada do Alto Uruguai e das Missões (URI) in Santo Ângelo, RS, Brazil.

Data analysis

Diversity data were measured as follows: (1) observed species richness (S_{obs}); (2) species richness estimated by rarefaction method (S_{rar}); (3) Shannon-Wiener heterogeneity index (H'); and (4) Smith-Wilson

evenness index (E_{var}). Of these, *H'* and E_{var} were calculated using the software Ecological Methodology (Krebs, 1999). Natural logarithm (ln) was used to calculate *H'*. For S_{rar}, all samples were standardized to 11 specimens, to nullify the effect of *N* (number of individuals) in species richness, using Biodiversity-Pro version 2 (McAleece *et al.,* 1997). The correlation among S_{obs}, S_{rar}, *H'*, E_{var} and *N* was tested by Linear correlation *r* in Past 1.34 (Hammer *et al.,* 2001).

Statistically significant differences in values of *H'*, E_{var}, S_{obs}, *N*, N_{exot} and N_{nat} between collection points were analyzed using the T test, in Past 1.34 (Hammer *et al.,* 2001). The preference of some species for a specific environment was tested with Wilcoxon tests based on their absolute abundances, using the same software.

The influence of space and time on assemblage diversity was estimated by the following calculation: $H'_{between} = H'_{total} - (Nj\ H'j)/Nt$; where $H'_{between}$ is the value of *H'* for a given component; H'_{total} is the value of *H'* considering all the samples together; *Nt* is the total number of individuals in all samples, *Nj* is the number of individuals in category *j*, *H'j* is *H'* within category *j*. Spatial (urban and rural zones) and temporal (monthly collections) components were considered.

The similarity between samples was investigated by cluster analysis using UPGMA method, in Past 1.34 (Hammer *et al.,* 2001). Similarity measures were Coefficient of Jaccard and Morisita index of similarity. Coefficient of Jaccard is a binary coefficient (deals with presence/absence data), so it was used to compare the similarities in species composition among samples. On the other hand, Morisita index deals with quantitative data, so it was used to compare samples in terms of relative abundance of each species. As the original Morisita index showed little differences between our samples, we used it after a logarithmic transformation [ln(x+1)], as recommended by Wolda (1981) and Krebs (1999) for communities with few species in common and many rare species, as the present sample.

RESULTS AND DISCUSSION

Species occurrence and abundances

In total, 13,379 drosophilids were analyzed (RZ: $N = 8{,}812$ and $S_{obs} = 25$; UZ: $N = 4{,}567$ and $S_{obs} = 16$), distributed as 26 species, 23 of which belonging to genus *Drosophila.* One species probably has not been described yet, and was called here *Drosophila* sp.Q2. This is the same species referred to by the same name by Gottschalk *et al.* (2007), in a study that reported its occurrence it in Morro da Lagoa da Conceição and Morro da Cruz, in Florianópolis, SC, and found abundantly by Sabrina C.F. de Oliveira in the Unidade de Conservação Ambiental Desterro (UCAD), also in Florianópolis (pers. comm.). The genera *Zygothrica, Zaprionus* and *Leucophenga* were represented by just one species each (Tables 1 and 2).

Two species of *Drosophila, D. aldrichi* and *D. repleta,* were recorded in the State of Rio Grande do Sul for the first time. For *D. aldrichi* this is the new southernmost record. With these new records, the number of described drosophilid species known for Rio Grande do Sul rises to 86. Also were found *D. nigricruria, D. virilis* and *Leucophenga maculosa,* which were just recently found for the first time in Rio Grande do Sul by Hochmüller *et al.* (2010).

From the 26 species found, six are exotic. Except for one collection in RZ, during the January of 2009, in summer, *D. simulans* was always the most abundant species in our study, showing expressive dominance in UZ (68% of the total of individuals) and being also the most abundant species in RZ (48% of the individuals). This species frequently is the most abundant exotic species in natural environments in Brazil (Sene *et al.,* 1980; Torres & Madi-Ravazzi, 2006; Schmitz *et al.,* 2007; Bizzo *et al.,* 2010; Hochmüller *et al.,* 2010). Its sibling species, *D. melanogaster,* is also commonly found in synanthropic environments, although with lower abundances, as in the present study. *Zaprionus indianus* is a recent invader (Vilela, 1999) and became a very abundant species in urbanized environments. Therefore, the abundance of *Z. indianus* in São Luiz Gonzaga seems to be comparatively lower than in other locations (Castro & Valente, 2001; De Toni *et al.,* 2001; Ferreira & Tidon, 2005; Silva *et al.,* 2005; Gottschalk *et al.,* 2007), where it represents, in some situations, more than half of collected individuals. In São Luiz Gonzaga, this species achieved a total relative abundance of about 5% in the urban zone and of about 1% in the forest fragment, similar to the results found by Hochmüller *et al.* (2010) in Cruz Alta, in the region of transition between the Atlantic Forest and Pampa Biome in the countryside of Rio Grande do Sul. The present study adds new evidence that the populations of this species are relatively small in this region. As this region is next to the southern limit of its distribution (Uruguay and northern Argentina), it is possible that this species is represented by marginal populations living in suboptimal conditions, limited by weather conditions like lower temperatures.

Another similarity between the drosophilids assemblages from São Luiz Gonzaga and Cruz Alta is the relatively higher representativeness of *D. immigrans* and *D. busckii,* when compared to other studies

TABLE 1: Monthly absolute abundance of drosophilid species collected in a forest patch in the rural zone (RZ) of São Luiz Gonzaga, RS, Brazil.

	SEP 07	OCT 07	NOV 07	DEC 07	JAN 08	FEB 08	APR 08	MAY 08	JUN 08	JUL 08	AUG 08	SEP 08	NOV 08	JAN 09
D. aldrichi							2							
D. antonietae			2						5					
D. bandeirantorum										1				
D. busckii	171	288	169							3		46		
D. buzzatii	4	8	2	2					4	2	2	2		
D. cardinoides									9	11	2	5		
D. griseolineata										5		3		
D. hydei	11	12	3	1					1		1	1		
D. immigrans	21	11	17		1		1		4		8	11	50	
D. maculifrons			1						6	5	6	4		
D. mediopunctata	3								3	2	1	2		
D. melanogaster	22	11				3	12		44		29	19		
D. mercatorum	139	98	97	3	8	2			27	60	83	58	44	1
D. nigricuria	2	5				2	2	3	9	6	8	10	2	1
D. onca									2		2	5	5	
D. pallidipennis			2		9	4			3		13	7	17	
D. paraguayensis											1			
D. polymorpha	41	12	18	8	70	34	8	3	118	182	335	101	24	100
D. simulans	187	290	207	20	45	52	52	5	204	347	1340	1275	56	146
D. sp. Q2			3										6	
D. virilis	2													
D. willistoni					10	31	6		32	101	8	6	1	626
Gr. *guarani* (females)									1					
Gr. *repleta* (females)	196	77	71	11	3	3	5	2	37	95	78	52	73	2
Gr. *tripunctata* (females)	7		1				1		2	1		3		
Leucophenga maculosa									33					
Zaprionus indianus							44		7	3	3	3	23	9
Zygothrica vittimaculosa		3							4	23	9			

TABLE 2: Monthly absolute abundance of drosophilid species collected in the urban zone (UZ) of São Luiz Gonzaga, RS, Brazil.

	SEP 07	OCT 07	NOV 07	DEC 07	JAN 08	FEB 08	APR 08	MAY 08	JUN 08	JUL 08	AUG 08	SEP 08	NOV 08	JAN 09
D. bandeirantorum											13			
D. busckii	123	90	8							7	8			
D. buzzatii	3	78	3	2		2			1					
D. cardinoides				1							1			
D. hydei	8	53	42							2	10		2	
D. immigrans	4		8						1		13	1	17	
D. maculifrons									6					
D. melanogaster	6	10	7		2		2	5	22	8	59	16		
D. mercatorum	61	36	16		6		3	3	10	10	45	3	5	2
D. nigricruria	1			2					2					
D. pallidipennis											1		1	
D. polymorpha	6	10	1	2	1	1			2	2	6	1		
D. repleta					2									
D. simulans	179	205	87	25	20	134	13	33	178	190	1401	367	86	171
D. willistoni						6								
Gr. *repleta* (females)	80	143	17	3	1			1	17	34	15	4	3	1
Gr. *tripunctata* (females)					1		1				8			
Zaprionus indianus					1	6	55	18	9	3	3		22	141

in Brazil. Contrasting *Z. indianus,* these species seem to be related to more temperate weather, becoming markedly rarer in northernmost localities (Ferreira & Tidon, 2005; Torres & Madi-Ravazzi, 2006; Gottschalk *et al.,* 2007; Schmitz *et al.,* 2007; Bizzo *et al.,* 2010). The other exotic species, *D. virilis,* is not commonly attracted to banana-baited traps and was represented by one individual only.

Between the Neotropical species, the most common were *D. mercatorum, D. hydei* and *D. buzzatii* in UZ (all belonging to *D. repleta* group) and *D. mercatorum, D. polymorpha* and *D. willistoni* in RZ. The abundances of the species of the *D. repleta* group are underestimated, since discrimination of females is difficult and just the males were identified. However, assuming that the relative abundances of the females were the same as of the males, *D. mercatorum* is the most common Neotropical species in São Luiz Gonzaga. This differentiates the assemblages of drosophilids collected in São Luiz Gonzaga from the assemblages found in Porto Alegre and in localities of Atlantic Forest and Amazon Biomes, where *D. willistoni* is almost always the most abundant Neotropical species (Martins, 1987; Silva *et al.,* 2005; Gottschalk *et al.,* 2007). Again, the results of the present study are similar to the findings by Hochmüller *et al.* (2010) in Cruz Alta, where a lower representativeness of *D. willistoni* was observed. On the other hand, in Cruz Alta *D. mercatorum* did not achieve expressive abundances (*D. maculifrons* was the most abundant Neotropical species). A high representativeness of *D. mercatorum* was found by Ferreira & Tidon (2005), in Brasília, Cerrado Biome, where it also was the most abundant Neotropical species.

Some important absences can be noticed in assemblages of drosophilids in São Luiz Gonzaga, like *D. malerkotliana, D. paulistorum* and *D. saltans* species group, taxa that are quite common in most part of Brazil. *Drosophila malerkotliana* (an introduced species) and *D. saltans* species group also seem to be absent in Cruz Alta, while *D. paulistorum* is present at low abundance in that locality (Hochmüller *et al.,* 2010).

The preference of some species for a given environment has been reported by many authors (Dobzhansky & Pavan, 1950; Sene *et al.,* 1980; Ferreira & Tidon, 2005; Tidon, 2006). In the present study, 16 (60%) out of the 26 species were found exclusively or preferentially in the forest, while nine did not express any preference and just one was exclusive of the city (Table 3). This last case was *D. repleta,* which in spite of being a Neotropical species, was introduced in many regions around the world, being currently a cosmopolitan species, normally associated to anthropic presence. The preference for the forest patch was higher among the Neotropical species, 70% of which occurring exclusively or preferentially in this environment; however, when only the introduced species are considered, this proportion decreased to one third.

TABLE 3: Drosophilidae species collected in São Luiz Gonzaga, RS, classified according to environment preference. Species without preference were abundantly present in both areas.

Only in forest	**Without preference**
D. aldrichi	*D. bandeirantorum*
D. antonietae	*D. busckii*
D. griseolineata	*D. buzzatii*
D. mediopunctata	*D. cardinoides*
D. onca	*D. hydei*
D. paraguayensis	*D. maculifrons*
D. sp.Q2	*D. melanogaster*
D. virilis	*D. simulans*
Leucophenga maculosa	*Zaprionus indianus*
Zygothrica vittimaculosa	**Only in city**
Preferentially in forest	*D. repleta*
*D. immigrans**	
*D. mercatorum***	
*D. nigricruria***	
*D. pallidipennis**	
*D. polymorpha****	
*D. willistoni***	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Diversity measures

The highest diversity was found in RZ, considering either heterogeneity (H') or species richness (S_{obs} or S_{rar}) (Table 4). Avondet *et al.* (2003), Gottschalk *et al.* (2007) and Garcia *et al.* (2012), in studies performed in the cities of Oxford, OH, USA, Florianópolis, SC, Brazil and Porto Alegre, RS, Brazil, respectively, found some differences in the abundance of species along an urban gradient, but did not find any decrease in diversity. On the other hand, other studies like those of Goñi *et al.* (1997), Ferreira & Tidon (2005) and Hochmüller *et al.* (2010), respectively, in Montevideo, Uruguay, Brasília, DF, Brazil and Cruz Alta, RS, Brazil, found some evidence of decrease in diversity in urbanized regions, when compared with natural environments. The factors that cause the decrease in diversity in some localities and not in others remain to be elucidated. Gottschalk *et al.* (2007) suggested that the existence of green areas nearby the urban areas could support the survival of native drosophilid species in the city. Considerable portions of natural environment remnants still persist in Florianópolis and, to a lesser extent, in Porto Alegre. On the other hand, the region where Cruz Alta and São

TABLE 4: Monthly variation in Shannon-Wiener heterogeneity index (*H'*), Smith and Wilson's index of evenness (E_{var}), observed species richness (S_{obs}), species richness estimated by rarefaction (S_{rar}, for n = 11), number of individuals (N), number of individuals of exotic species (N_{exot}) and number of individuals of Neotropical species (N_{nat}), of the assemblages of drosophilids in urban (UZ) and rural (RZ) zones of São Luiz Gonzaga, RS, Brazil.

		SEP	OCT	NOV	DEC	JAN	FEB	APR	MAY	JUN	JUL	AUG	SEP	NOV	JAN
E_{var}	ZU	0.214	0.471	0.332	0.433	0.461	0.218	0.337	0.525	0.243	0.280	0.163	0.127	0.263	0.148
	ZR	0.218	0.248	0.175	0.467	0.311	0.320	0.318	0.963	0.300	0.193	0.158	0.208	0.327	0.102
H'*	ZU	1.318	1.569	1.426	0.820	1.170	0.445	0.749	1.048	0.911	0.644	0.504	0.252	1.066	0.722
	ZR	1.649	1.366	1.408	1.137	1.269	1.387	1.443	1.066	1.921	1.530	0.941	0.832	1.902	0.850
S_{obs}**	ZU	9	7	8	5	7	5	5	4	9	7	11	5	6	3
	ZR	11	10	12	5	6	7	9	3	18	14	17	17	10	6
S_{rar}**	ZU	3.57	4.48	4.08	3.07	3.78	1.96	2.58	3.11	2.97	2.38	2	1.51	3.26	2.07
	ZR	4.49	3.65	3.78	3.55	3.63	3.8	4.04	3	5.21	4.18	2.82	2.68	5.43	2.73
N**	ZU	391	482	172	32	32	149	73	59	231	222	1560	388	133	214
	ZR	603	738	521	34	143	128	127	11	515	751	1851	1558	228	883
N_{exot}	ZU	312	305	110	25	23	140	70	56	210	208	1484	384	125	212
	ZR	403	600	393	20	46	55	109	5	259	353	1380	1354	129	155
N_{nat}*	ZU	79	177	62	7	9	9	3	3	21	14	76	4	8	2
	ZR	200	138	128	14	97	73	18	6	256	398	371	204	99	728

* $p < 0.05$, ** $p < 0.01$; all measures with significant differences are higher in RZ in comparison with UZ.

Luiz Gonzaga are located is characterized by a highly human-modified landscape, with few and small patches of natural vegetation. Some authors point out that local biodiversity may be affected by the regional amount of remnant vegetation, with a fragmentation threshold below which diversity becomes dependent of patch size (Pardini *et al.,* 2010).

Here, no significant difference between UZ and RZ in the abundance of exotic species (N_{exot}) was observed, but the number of specimens of endemic species from Neotropics (N_{nat}) was significantly lower in UZ (Table 4). This suggests that the forest patch in RZ can be easily invaded by at least some exotic species, probably because it is a small fragment of forest, in a region quite fragmented by agricultural cultures. On the other hand, for most Neotropical species, it is difficult to survive in face of the expansion of urban environments over natural ones.

In general, the observed species richness (S_{obs}) was higher in the period between June and November (roughly winter and spring), varying from 5 to 11 in UZ and from 10 to 18 in RZ, while it was lower from December to May (roughly summer and autumn), with 3 to 7 species in UZ and 3 to 9 in RZ (Table 4). A higher richness in drosophilid assemblages during winter was also found by Torres & Madi-Ravazzi (2006) in the state of São Paulo. In São Luiz Gonzaga, a severe dry and hot period during summer may have caused a negative effect on drosophilidae diversity.

The expressive abundance of *D. simulans* seemed to affect the indexes of heterogeneity (*H'*) and evenness (E_{var}) of the assemblage. The highest value of E_{var} was found in May 2008 (E_{var} RZ = 0.963 and E_{var} UZ = 0.525), period of autumn (Table 4), when the species richness and the relative abundance of *D. simulans* were lower in comparison to other months, which was observed again in summer periods. In RZ, the highest heterogeneity was observed during June 2008 (*H'* = 1.921), autumn, as opposed to the findings by Benado & Brncic (1994), in Chile, in a study that reported the lowest diversity in the same period. In UZ, the highest heterogeneity was found in October 2007 (*H'* = 1.569), spring, as found by De Toni *et al.* (2007) in Santa Catarina. The lowest diversity was found in both sites in September of 2008, winter, with *H'* = 0.252 in RZ and *H'* = 0.832 in UZ. This low diversity is due to the high dominance of *D. simulans.* The dominance of one species acting negatively on community diversity was noticed by De Toni *et al.* (2007) and Brncic *et al.* (1985), with a large dominance of *D. willistoni* and *D. simulans* in their collections, respectively.

Table 5 shows the correlations between the diversity measures in each site. In both sites, S_{obs} showed positive and significant correlation with *N,* while S_{rar} was correlated with *H'.* In UZ, *H',* E_{var} and S_{rar} showed a statistically significant positive correlation, while in ZR, E_{var} was negatively correlated with S_{obs} and *N.*

In spite of the interference caused by the dominance of *D. simulans,* the time component was a determinant in the diversity of the assemblage, although more than half of the diversity could not be explained by the analyzed components (Table 6). The time component in the present study showed a relatively high contribution to the diversity in comparison with other similar studies (Silva *et al.,* 2005; Gottschalk *et al.,* 2007; Schmitz *et al.,* 2010). These studies, however, performed just seasonal collections, and studies based on monthly collections, like the present one, are not

TABLE 5: Linear correlation *r* between *H', Evar,* S_{obs}, S_{rar} and *N* in the drosophilid assemblages of urban (bottom left) and rural (top right) zones of São Luiz Gonzaga, RS, Brazil.

	H'	E_{var}	S_{rar}	S_{obs}	*N*
H'		-0.055726	0.98521***	0.20562	-0.44076
E_{var}	0.59474*		-0.067533	-0.55521*	-0.55744*
S_{rar}	0.97521***	0.68576**		0.19631	-0.43809
S_{obs}	0.23405	-0.1764	0.25631		0.71515**
N	-0.22535	-0.417	-0.25384	0.64962*	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

common. These results stress the great effect of the temporal changes in drosophilid assemblages that, especially in areas with a marked seasonal regime, as the Pampas, surpass the spatial effects, even when contrasting environments are compared.

Similarity analysis

In general, the present results show that species composition, as measured by the Jaccard coefficient (Fig. 2), is markedly affected by seasonality, being more similar in the colder months of the year. All samples taken between June and October (late autumn, winter and early spring) clustered together within a group sharing at least 40% of the species, while all the samples from January to May (summer and early autumn) lied outside this group. The months of November and December (late spring) seem to be a transition period, as some samples clustered in the coldest period and other samples in the hottest period. The environment (urban or forested) was also shown to be an important factor, since some clustering between sites can be observed, but to a lesser extent than temporal factors.

The Morisita index showed that when the structure of the assemblage is considered, the effect of seasonality is evident, since samples clustered into similar groups in consecutive periods and in the same season, showing the seasonal preference of some species again (Fig. 3). Almost all samples taken showed a high abundance of *D. simulans,* so the clustering was more influenced by secondary species. The first group of samples to split off the cluster is composed roughly by samples collected in hot months in the urban zone, and can be characterized by a relatively high abundance of *Z. indianus.* The second group to split is constituted exclusively by samples from the forest patch, marked by a higher relative abundance of *D. polymorpha.* Some summer samples within this group formed a subcluster with, besides *D. polymorpha,* a higher representativeness of *D. willistoni.* The remaining samples, mainly urban samples, but also

TABLE 6: Contribution of temporal and spatial components to the diversity in the assemblages of drosophilids in São Luiz Gonzaga, RS, Brazil.

	H'	%
Temporal	0,3991	25,4
Espacial	0,0906	5,77
Not explained	1.0815	68.83
Total	1.5713	100

several samples from the forest patch, are those that showed the higher dominance of *D. simulans.* Among them, a group of samples collected in spring clustered together and have in common a relatively high abundance of *D. busckii* and *D. mercatorum.*

Concluding remarks and future directions

Studies that compared drosophilids assemblages in forested areas with urban ones have consistently found marked differences in relative species abundances, but not always in diversity (Avondet *et al.,* 2003, Gottschalk *et al.,* 2007). However, the present study is the second recent survey to strongly suggest a marked biodiversity loss with the expansion of urban landscapes in detriment of natural ones in the countryside of the state of Rio Grande do Sul. Hochmüller *et al.* (2010) found lower species richness in the urban area of Cruz Alta, when compared to a forest remnant nearby. The present study found the same pattern in São Luiz Gonzaga. Additionally, we could detect a reduction in diversity also when it was measured by Shannon-Wiener index and species richness by rarefaction. It is notable too that 70% of the Neotropical species showed a preference for the forest fragment, with a significant reduction in abundance of native species in the city, reinforcing the importance of natural environments to maintain the regional biodiversity. Similar results were relatively well documented in the Cerrado biome (Ferreira & Tidon, 2005, Tidon, 2006, Mata *et al.,* 2010), where it was also verified that many Neotropical species that occur in natural environments were absent in the city, while others decreased in abundance as the degree of urbanization increased. The Cerrado and the Pampa biomes are similar in being constituted by natural formations of forests inserted in a landscape dominated predominantly by savanna-like environments. Future studies, especially in the Pampa, could indicate if the patterns of response of the biodiversity to landscape modification are similar in the two biomes.

The forest remnant surveyed in the present study is a very small and disturbed fragment, inside an

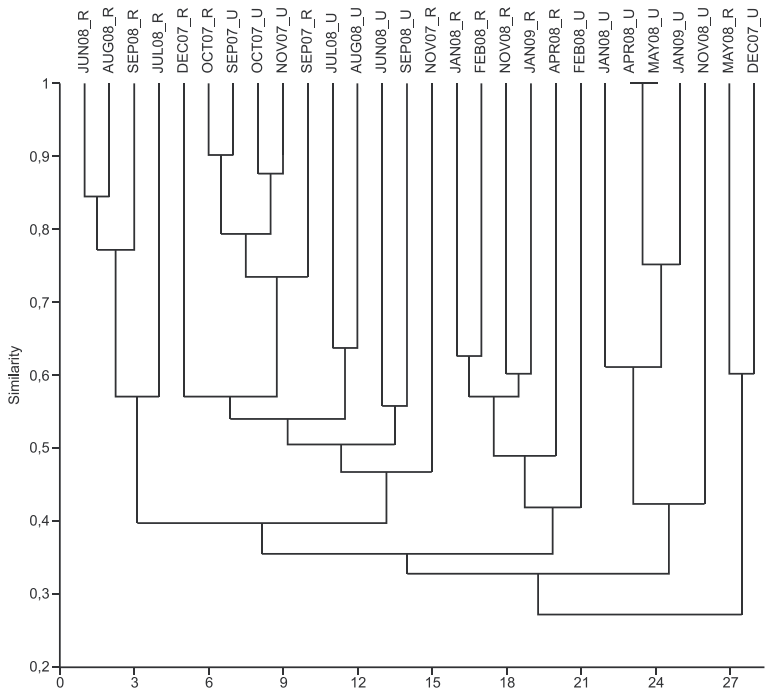

FIGURE 2: UPGMA dendrogram showing the similarity in species composition of monthly samples of drosophilids in urban (U) and rural (R) zone in São Luiz Gonzaga, RS, Brazil, according to Jaccard similarity index.

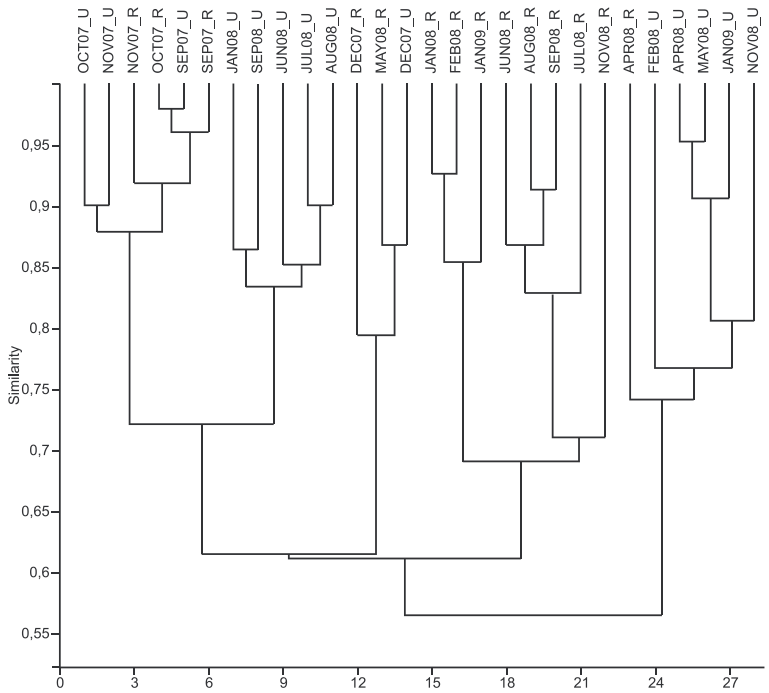

FIGURE 3: UPGMA dendrogram showing the similarity in species abundances of monthly samples of drosophilids in urban (U) and rural (R) zone in São Luiz Gonzaga, RS, Brazil, according to Morisita index.

agricultural landscape, and, as we noticed, although still a refuge for Neotropical species absent in the city, is also highly invaded by some introduced species, bioindicators of disturbed environments. Considering that the assemblages of drosophilids in undisturbed natural environments of the Pampa are still completely unknown, future studies are needed to assess the portion of the biodiversity that can have already

been lost in a landscape widely converted to agricultural fields. Although the impact of the urbanization on the natural assemblages is relatively well studied, the effects of the change of land use to agriculture and cattle raising are still little known.

RESUMO

O Pampa brasileiro (extremo sul do país) está, atualmente, vastamente modificado devido ao aumento das atividades agrícolas. Em muitos lugares, apenas pequenos fragmentos de campo permanecem em uma paisagem agrícola. Drosophilidae (Diptera) tem sido amplamente utilizadas como bioindicadores para monitorar os efeitos das mudanças antropogênicas em ambientes naturais. Porém, a fauna de Drosophilidae no Bioma Pampa de ambientes naturais ou perturbados, ainda permanece amplamente desconhecida. O presente estudo é uma das primeiras tentativas de preencher esta lacuna, apresentando resultados de coletas mensais no município de São Luiz Gonzaga (28°24'28"S, 54°57'39"W), no Pampa brasileiro. Um inventário de espécies foi conduzido em dois ambientes contrastantes, uma zona urbana e um remanescente de floresta (zona rural). Em ambos os locais, armadilhas com banana fermentada foram usadas para capturar drosofilideos adultos. A identificação foi feita através da morfologia externa e da terminália dos machos. No total, 13,379 drosofilideos foram analisados (zona rural: $N = 8{,}812$ *and* $S_{obs} = 25$*; zona urbana:* $N = 4{,}567$ *and* $S_{obs} = 16$*). No presente estudo, 16 (60%) das 26 espécies coletadas foram encontradas exclusivamente ou preferencialmente no fragmento de mata. O período de maior riqueza foi entre os meses de junho a novembro (inverno-primavera), e o período de menor riqueza foi de dezembro a maio (verão-outono). Uma análise de cluster pelo Coeficiente de Jaccard mostrou que a composição da assembléia muda ligeiramente quando o período do ano com temperaturas mais elevadas (janeiro-maio) é comparado com o período de temperaturas menos elevadas (junho-outubro). A abundância das espécies foi também altamente afetada pela sazonalidade, como revelou o Índice de Morisita, onde as amostras foram agrupadas em períodos consecutivos dentro de uma mesma estação, mostrando a preferência sazonal de algumas espécies. O componente tempo foi determinante na diversidade da assembléia, superando o efeito espacial. A forte redução na diversidade na área urbana quando comparada com o pequeno fragmento de floresta, torna evidente a importância do ambiente natural para a preservação da diversidade no bioma Pampa, atualmente com sua paisagem altamente alterada.*

Palavras-Chave: Bioma Pampa; Drosophilidae; Diversidade; Bioindicador.

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