Drosophilid assemblages as a bioindicator system of human disturbance in the Brazilian Savanna

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Abstract

According to the Convention on Biological Diversity (CBD) the development of bioindicators is extremely necessary to achieve the conservation targets by 2010, and insects are considered an effective group for this goal. Drosophilids are regarded as potential indicators, although this idea remains untested. Therefore, we followed up a protocol to test the drosophilid potential indicator for human disturbance in the Brazilian Savanna, one of the richest and most threatened tropical biomes in the world. Sampling was undertaken in one urban environment and two biological reserves, representing four habitat types (undisturbed gallery forest, disturbed gallery forest, undisturbed savanna, and urban environment). We examined differences in the drosophilid assemblages among habitat types and used the Indicator Value (IndVal) method to point out the indicator species. We also tested the two-stage indicator validation, a protocol recently proposed in the literature, to validate the indicator species for undisturbed gallery forest and savannas, in independent samples. The assemblage variables varied mainly in undisturbed gallery forests, and reflected changes from an undisturbed to a disturbed stage. The IndVal associated with the two-stage protocol showed reliable characteristic species, which are very helpful for diagnostic surveys. Likewise, species that can detect changes in the habitats were also found. We found a set of indicators, which together may be very efficient for both assessing and reflecting a variety of conditions, improving the confidence of the bioindication system, expanding the taxonomic options for bioindicators, and therefore, contributing to the conservation of this region.

Keywords: Cerrado biome, Characteristic species, Conservation biology, Detector species, Drosophila, Indicator value, Monitoring, Zaprionus indianus .

Introduction

The Convention on Biological Diversity (CBD) has established a set of targets to reduce the rate of global biodiversity loss by the year 2010. The development of indicators is among the strategic plans for assessing and monitoring progress toward these conservation targets (United Nations *2002*). Despite bioindicators being considered a relatively inexpensive and readily applied approach to assessing environmental condition (Hilty and Merenlender *2000*; Dale and Beyeler *2001*), bioindicators of disturbance have only been developed for select geographic areas, and using a fairly narrow range of taxa (McGeoch *2007*). Ironically, this is particularly true for tropical areas where rates of habitat destruction and biodiversity loss are amongst the highest in the world (Dobson *2005*).

The use of species or groups of species to reflect the condition of the environment or a component of biodiversity is far from new. However, only fairly recently have rigorous methodologies been developed and adopted for the identification of bioindicators (McGeoch *1998*, *2007*), and significant progress in the theoretical and methodological development of bioindicators has recently been made (Landres et al. *1988*; Noss *1990*; Caro and O'Doherty *1999*; Hilty and Merenlender *2000*). It is strongly recommended that the

selection of an indicator must be supported by a sound conceptual framework and follow a scientifically rigorous protocol (Niemi and McDonald *2004*). This process should include a clear definition of the bioindication objectives and the scale at which the study is to be conducted, the fulfillment of the taxon in a priori selection criterion, the establishment of the relationships between the indicators and the environment, and the development and testing of hypotheses (McGeoch *1998*). In addition, a two-stage process (quantitative identification and verification) is necessary to establish the degree of confidence with which the bioindicator may be applied (McGeoch et al. *2002*). The development of a suite of indicator variables (bioindicator system), rather than a single indicator (e.g., one species; Hilty and Merenlender *2000*) is also recommended to increase the reliability of a bioindication system. These recommendations along with new methodologies are considered a substantial improvement on bioindication selection efficiency and are also likely to increase the successful adoption of bioindicators as management tools. Despite this, to date only a few studies have adopted these recommendation and tested the new methodologies (McGeoch *2007*).

Insects are regarded as an effective group for bioindication (Brown *1991*, *1997*; Kremen et al. *1993*; McGeoch *1998*; Hilty and Merenlender *2000*; Andersen *2004*; Samways *2005*), and have indeed played an important role in the development and progress in this field of investigation (McGeoch *2007*). However, despite their known sensitivity to environmental condition, studies involving insects rarely include a verification of proposed bioindicators, and there remains a narrow range of bioindicator scenarios and geographic regions for which insect bioindicators have been developed (McGeoch *2007*). Drosophilids, for example, are potentially good taxa for bioindicators, and certainly no other insect group has been as thoroughly studied (Powell *1997*). Although the potential of these flies for bioindication has already been suggested (Parsons *1991*, *1995*; Saavedra et al. *1995*; Ferreira and Tidon *2005*) this assumption remains untested.

This paper thus proposes a bioindicator system of human disturbances for the Brazilian Savanna, locally known as Cerrado biome, based on the drosophilid assemblages that inhabit it. The Cerrado is considered one of the 25 hotspots of the world (Myers et al. *2000*) due to its high conservation value and the disturbance threat that it faces. It has been extensively transformed into urban and rural environments (Klink and Machado *2005*), and only around 2% of its area is currently protected (Silva et al. *2006*). In fact, Cerrado conservation has not reached the status of tropical forests, such as the Amazon and Atlantic Rainforests (Marris *2005*). Therefore, studies aiming at developing tools for the diagnosis, monitoring, and management of biodiversity conservation for this biome are urgently needed. Here, we evaluate a protocol proposed in the current literature for testing the potential indicator value of drosophilids for different habitat types in the Cerrado biome. In this context, the main goals of the present study were: (1) to develop a set of indicator variables of undisturbed and disturbed Cerrado habitats, including variables (i) at the assemblage level (richness and abundance of neotropical, exotic, widespread, and narrow range species), and (ii) at the species level (different species); and (2) to test the two-stage indicator validation process (McGeoch et al. *2002*) in two contrasting environments of the biome: savannas and gallery forests.

Methods and data

Study area, sampling, and species identification
The Cerrado biome is a complex of seasonal savannas that covers most of the interior of Brazil, and is the second largest Brazilian biome, exceeded only by the Amazon Forest (Ratter et al. *1997*). This biome, which includes forest, woodland, savanna, and grassland habitats, demonstrates high natural heterogeneity due to the interaction between seasonality, topography, edaphic features, and climate fluctuations (Oliveira and Marquis *2002*). Climate in the Cerrado is tropical dry winter (Aw in the Koeppen system) in 95% of the

biome, changing to cooler Cw at higher altitudes, and precipitation is highly seasonal, characterized by a well-defined dry season from May to September (Oliveira and Marquis *2002*).

Sampling was undertaken in one urban environment and two biological reserves, including four habitat types: undisturbed gallery forests, disturbed gallery forests, undisturbed savannas, and urban environment. The drosophilids were caught using a trap developed to minimize bias in capturing different species of flies attracted to banana baits (Medeiros and Klaczko *1999*), monthly, for 12 months (Table 1). The urban environment that was sampled was Brasilia, the capital of Brazil, located in the heart of the Cerrado (15°47' S; 47°57' W). Three sites were sampled in this urban environment; in each one-fifteen traps were positioned 10 m from each other, along a 150 m transect. Both biological reserves studied here are situated in the outlying neighborhoods of Brasilia. The National Park (NP) is located 10 km Northwest of Brasilia (15°40' S; 47°54' W), and covers an area of 30,000 ha. The Ecological Reserve of IBGE (Instituto Brasileiro de Geografia e Estatística) (RECOR), located 35 km south of Brasilia (15° 56' S; 47° 53' W), is part of an environmental protection area that covers 10,000 ha. This last reserve was sampled in two different periods, named RECOR I and RECOR II (Table 1).

Table 1
Characterization of the sampling areas and sites

Area	Sampling period	Habitat types	Number of sites	Number of traps	No. months samples	Source
Brasilia National Park (NP)	07/1999 to 06/2000	Undisturbed savanna	1	10	10	Tidon (*2006*)
		Undisturbed gallery forest	1	10	10	
Ecological Reserve of IBGE (RECOR)	I. 07/1999 to 06/2001	Undisturbed savanna	1	10	10	Tidon (*2006*)
		Undisturbed gallery forest	1	10	10	
	II. 04/2001 to 05/2002	Undisturbed savanna	6	1	6	Mata (*2002*)
		Undisturbed gallery forest	3	1	3	
		Disturbed gallery forest	3	1	3	
Brasilia City (BsB)	09/2000 to 08/2001	Urban environment	3	15	45	Ferreira and Tidon (*2005*)

In NP and RECOR I, one gallery forest and one savanna-like vegetation (locally known as cerrado sensu stricto or just "cerrado") area were sampled, encompassing four undisturbed sites. Ten traps were placed in each site, positioned 10 m from each other, along a 100 m transect. In the RECOR II sample, 12 sites (one trap per site) located in a variety of vegetation forms (disposed at least 100 m from each other) were classified into three habitat types: (1) undisturbed gallery forest, (2) disturbed gallery forest, and (3) undisturbed savanna.
The drosophilid specimens were identified by identification keys, species description and, in some cases, the male terminalia (Freire-Maia and Pavan *1949*; Frota-Pessoa *1954*; Val *1982*; Vilela *1983*, *1992*; Vilela and Bächli *1990*).

Drosophilid assemblages as indicators

First, species were classified into categories based on their biogeographical origin (neotropical or exotic species), and distribution pattern (widespread or narrow range species). The widespread species were those that occurred in all 19 sites; the remaining species were classified as narrow range because they have occurred in <16 sites. The following assemblage variables were analyzed: (1) abundance of neotropical species, (2) abundance of exotics, (3) abundance of narrow range species, (4) abundance of widespread species, (5) neotropical species richness, (6) exotic species richness, and (7) narrow range species richness. Generalized Linear Models (GLM; McCullagh and Nelder *1989*) were used to determine significant differences ($\alpha < 0.05$) between assemblage variables among the four habitat types. A Poisson error distribution was assumed for species' richness and abundance variables, and deviance was used as a measure of goodness-of-fit (Collett *1991*).

Drosophilid species as indicators

To identify indicator species characteristic of particular habitats we used the Indicator Value (IndVal) method, developed by Dufrene and Legendre (*1997*), which combines measurements of the degree of specificity of a species to an ecological state (for instance, a habitat type), and its fidelity (or frequency of occurrence) within that state. It provides an indicator value (IndVal) for each species, as a percentage, based on two criteria:

$$\text{specificity measure:} A_{ij} = N individuals_{ij} / N individuals_i \tag{1}$$

where Nindividuals $_{ij}$ is the mean number of species i across sites of group j, and Nindividuals $_i$ is the sum of the mean numbers of individuals of species i over all groups;

$$\text{fidelity measure:} B_{ij} = N sites_{ij} / N sites_j \tag{2}$$

where Nsites $_{ij}$ is the number of sites in cluster (habitat) j, where species i is present, and Nsites $_j$ is the total number of sites in that cluster. The percentage indicator value for species i in cluster (habitat) j is then:

$$IndVal_{ij} = A_{ij} \times B_{ij} \times 100$$

The IndVal represents a significant methodological advance in bioindication studies, and it has various advantages over other analyses used for finding indicator species (McGeoch and Chown *1998*). Using this method, it is possible not only to identify characteristic species (Dufrene and Legendre *1997*), which are essential for the bioindication surveys, but also detector species, which may be more useful in monitoring changes in environmental condition (McGeoch et al. *2002*).

Characteristic species

Characteristic species are those that have both high specificity and fidelity components of the IndVal measure in a particular habitat type. Therefore, they will have a high percent IndVal. These species make reliable indicator species not only because they are specific to a locality, but also because they have high probability of being sampled in that locality during monitoring and assessment (McGeoch and Chown *1998*).

First, we sought to obtain a general overview of the species habitat preferences, and we merged the whole data set into a single matrix (NP + Recor I + Recor II + Brasilia city), classifying the four habitat types as undisturbed gallery forest (gf), undisturbed savanna (sv), disturbed gallery forest (dgf), and urban environment (ur). Species with significant Indicator Values higher than 70% in a site type were then

regarded as characteristics species of that particular habitat (see also van Rensburg et al. *1999*). Here, the random reallocation procedure was used to test the significance of the IndVal measures for each species (Dufrene and Legendre *1997*).

Detector species

McGeoch et al. (*2002*) predicted that because characteristic species are highly specific and restricted to a single habitat, they are likely to be vulnerable to change in their preferred habitat state, and may eventually have little value for monitoring. On the other hand, species presenting moderate specificity levels for one habitat state and occurring across a range of habitat states, are more likely to move to adjacent habitats under changing habitat conditions than are highly specific, characteristic species. Thus, such 'detector species' may indicate the direction of change, as well as being of more sustainable use in monitoring (Van Rensburg et al. *1999*; McGeoch et al. *2002*). Because the Cerrado biome has been intensively transformed by human activities (Klink and Machado *2005*), it represents an excellent opportunity for testing the original prediction for detector species. Specifically, when undisturbed forests are subjected to disturbance (i.e., vegetation cut), their canopies become more open, and their environment thus more similar to that of savanna habitat (Pivello and Coutinho *1996*). Based on the rationale in McGeoch et al. (*2002*), under such a change, characteristics species are likely to decline in abundance (and thus in fidelity), whereas detector species abundance is predicted to increase. Thus, drosophilid species with moderate preference for savannas (detector species) would be more likely to invade gallery forests in early stages of their change to a more open state. To test this prediction, we compared the species IndVals among undisturbed gallery forests, disturbed gallery forests, and savannas. Species with moderate preference for savannas were identified as those with IndVals between 50% and 70% for this habitat, and between 5% and 49% for undisturbed gallery forests. Similarly, detector species for disturbed gallery forests should have IndVals larger than those for undisturbed gallery forests (McGeoch et al. *2002*).

We also tested a second prediction, proposed in this study, that assemblages associated with undisturbed habitats would change under increasing disturbance, with some species increasing their preferences for the disturbed habitats, regardless of their moderate preference for a specific habitat type. This prediction was tested by comparing the following habitat types: (1) undisturbed gallery forests × disturbed gallery forests; (2) undisturbed gallery forests × urban environments; and (3) undisturbed savannas × urban environments. For the second prediction, species whose IndVal (>50%) were higher in disturbed habitats in comparison with undisturbed ones, regardless of their moderate preference for a specific habitat type (savanna), were also identified as possible detector species.

Two-stage indicator validation

The indicator species identified here were tested following the protocol suggested by McGeoch (*1998*) and McGeoch et al. (*2002*). In this approach, after initial bioindicator identification, the proposed bioindicators must be tested by re-sampling the same environment under different temporal or spatial conditions: periods with different climatic conditions, or another place in the region where the bioindicator will be used. Here, the independent samples used were (1) PN plus RECOR I, and (2) RECOR II (Table 1). In this investigation, we included only the undisturbed gallery forests (five sites) and undisturbed savannas (eight sites), using two strategies. In the first one, data of PN plus RECOR I were used to generate the first set of characteristic species for those habitat types, a process called bioindicator identification. They were then tested following the same approach, but now using an independent set of samples, i.e., RECOR II (Table 1), which corresponds to the process of validation. In the second strategy, the complete data set (PN + RECOR I + RECOR II) was used, and the samples were submitted to random selection. The first, randomly selected half of the samples were used to generate a set of characteristic species (identification), and the second

half was used to test them (validation). It was expected that the most reliable indicators would be identified and re-identified by both strategies. Due to the low number of observations in PN and RECOR I, the criteria for selection in this two-stage process was the frequency with which the species' had IndVals higher than 70%, rather than the significance level. However, the significance level of the IndVals was used to assess the robustness of the indicators.

Results

Amongst 77,286 individuals analyzed here, 35 neotropical and six exotic species (Drosophila immigrans, D. malerkotliana, D. melanogaster, D. simulans, Scaptodrosophila latifasciaeformis, and Zaprionus indianus) were identified. Six species (D. cardini, D. mercatorum, D. nebulosa, D. simulans, D. sturtevanti, and Z. indianus) occurred at all 19 sites, and were classified as widespread species. The other 35 species were considered narrow range species in the context of the study area (Appendix 1).

Drosophilid assemblages as indicators

There were significant differences between habitats for two of the four abundance variables, i.e., the abundances of neotropical and narrow range species (Table 2). Drosophilid abundances were generally higher in undisturbed forest than in disturbed forest, although there were no significant differences in the pairwise comparisons (Table 2, Fig. 1a, b). All richness variables varied significantly among habitat types (Table 2). Undisturbed forests had the highest mean richness of both neotropical and narrow range species (Fig. 1c, d). The urban environments had the highest mean richness of exotic species and the lowest neotropical species richness (Fig. 1c, e).

Fig. 1
Means and 95% confidence intervals of the variables that showed significant differences among habitat types (significant Wald statistic values). gf, undisturbed gallery forest; dgf, disturbed gallery forest; sv, undisturbed savanna; ur, urban environment. Different letters indicate significant pairwise differences between habitats
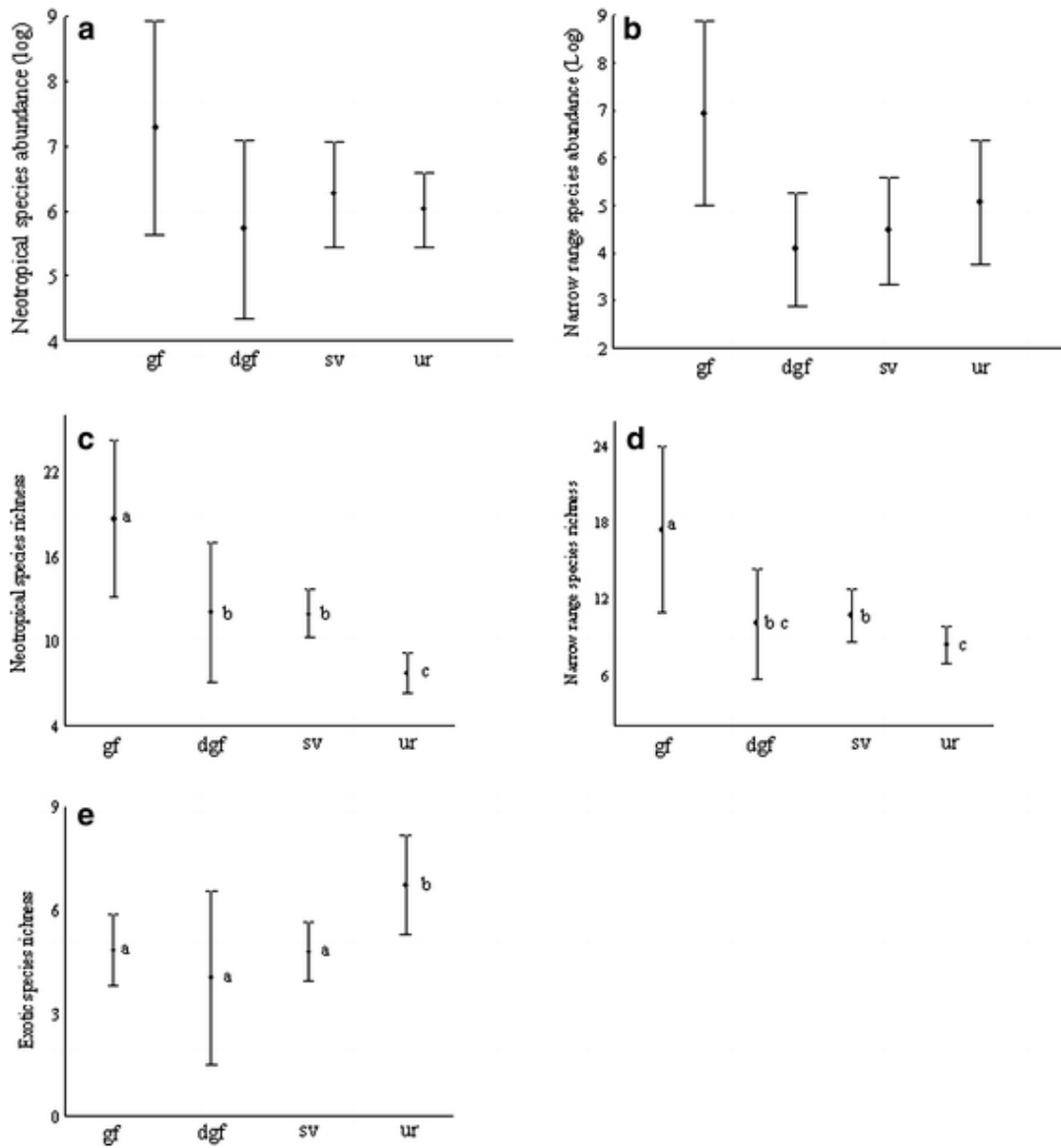

Table 2
Wald's statistics (W) obtained by the Generalized Linear Models (GLM) for seven assemblage variables, indicating which of them show significant differences among habitat types (undisturbed gallery forest; disturbed gallery forest; undisturbed savanna, and urban environment)

| Variables	df	Neotropical	Exotic	Narrow range	Widespread
Abundance (W)	3, 15	8.16*	4.46	10.57*	4.5
Richness (W)	3, 15	38.44**	12.71**	23.62**	

* P < 0.01
** P < 0.001

There were clear differences in the relative abundances of species categories among habitat types (Fig. 2). In terms of biogeographical origin, the undisturbed and disturbed forests were dominated by neotropical species, whereas savannas and urban environments were dominated by exotics (Fig. 2a). In terms of species distribution, the narrow range species (those that occurred in <16 sites) dominated undisturbed forests, while the widespread species became increasingly dominant from disturbed forests, to savannas and almost exclusively dominated the urban environments (Fig. 2b).

Fig. 2
Differences in drosophilid assemblage composition among habitat types, showing relative abundance of (a) neotropical and exotic species, and (b) narrow range and widespread species. gf, undisturbed gallery forest; dgf, disturbed gallery forest; sv, undisturbed savanna; ur, urban environment
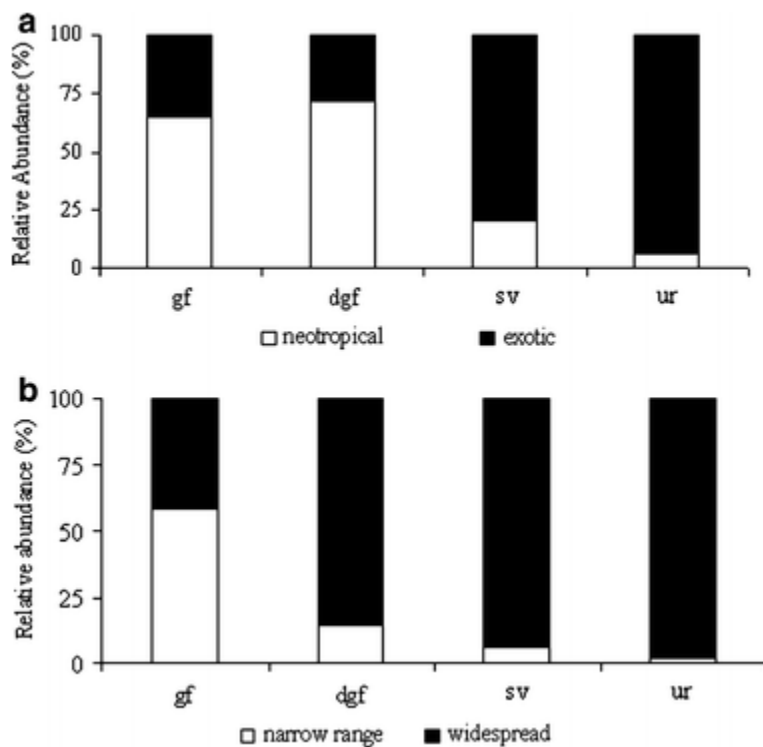
Drosophilid species as indicators

Characteristic species

Most of the habitat types, with the exception of disturbed forest, had species with high Indicator Values (Table 3). The majority of the species had highest IndVals for the gallery forests. Among the 24 species that preferred undisturbed gallery forests, five (Drosophila maculifrons, D. mediopunctata, D. ornatifrons, D. paraguayensis, and D. willistoni) had IndVals significantly higher than 70%, and can thus be considered characteristic (indicators) of this habitat. Drosophila cardinoides and D. melanogaster were identified as characteristic species of urban environments, and only D. nigricruria as characteristic of undisturbed savanna (Table 3). The analysis of the relationship between the two components of the IndVal, specificity and fidelity, clearly illustrates that the undisturbed gallery forests had not only more species with high specificity than the other habitat types, but also species filling most regions of the IndVal space (Fig. 3). There were no species with high specificity in disturbed gallery forest (Fig. 3), and only undisturbed gallery forest had species that were truly rare (i.e., highly specific with low fidelity (low occupancy or narrow range; Fig. 3).

Fig. 3
Relationships between fidelity and specificity (the two components of the Indicator Value) for species in the drosophilid assemblage in four habitat types
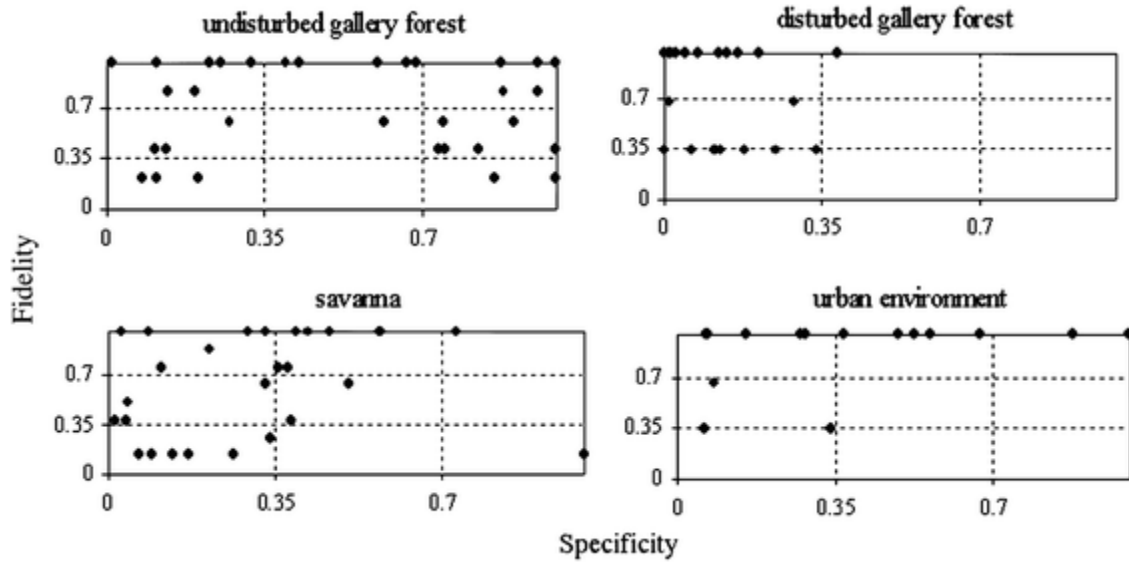

Table 3
Species Indicator Values (IndVal) showing which and how many species prefer each of the four habitats types

Species	IndVal (%)	Species	IndVal (%)
Undisturbed gallery forest		Disturbed gallery forest	
D. paraguayensis	100*	D. fuscolineata	38
D. willistoni	96*		
D. ornatifrons	88*	Undisturbed savanna	
D. maculifrons	77*	D. nigricruria	73*
D. mediopunctata	71*	D. hydei	57
D. malerkotliana	69	D. mercatorum	47
D. immigrans	67	D. nebulosa	40
D. mediostriata	61*	D. cardini	33
D. schildi	54*	D. fumipennis	13
D. guaru	45	D. medioimpressa	13
D. sturtevanti	43	D. mesostigma	13
D. ararama	40		
D. atrata	40	Urban environment	
D. bandeirantorum	40	D. melanogaster	100*
D. polymorpha	40	D. cardinoides	87*
D. neocardini	33	D. simulans	67*
D. bocainensis	30	Z. indianus	56
D. austrosaltans	29	D. prosaltans	53*
D. arauna	20	S. latifasciaeformis	53
D. neoguaramunu	20	D. busckii	49

Species	IndVal (%)	Species	IndVal (%)
D. onca	20	D. paranaensis	11
D. paramediostriata	20		
D. pallidipennis	17		
D. aragua	16		

* $P < 0.05$

Detector species

Drosophila hydei presented low specificity for undisturbed gallery forest (IndVal = 2%), moderate for savannas (IndVal = 61%), and an intermediate value (between undisturbed forest and savanna) in disturbed forests (IndVal = 20%). Therefore, only this species matched the original conditions for detector of changes from undisturbed to disturbed gallery forest, since it was more prevalent in an intermediate habitat (disturbed gallery forests) than in the less preferred habitat stage (undisturbed forest), and still had a moderate preference for the other habitat stage (savanna) (Table 4). Besides D. hydei, another seven species (D. busckii, D. immigrans, D. mercatorum, D. prosaltans, D. simulans, Scaptodrosophila latifasciaeformis, and Zaprionus indianus) met the second prediction for detector species of change from undisturbed to disturbed habitats. Those species increased their preferences (or IndVals) from undisturbed forests and savannas to urban environments, regardless of showing a moderate preference for savannas, as expected by the original prediction (Table 4).

Table 4
Indicator values of those species that matched the criteria for detector species for each comparison. (1) Undisturbed gallery forest (gf) × disturbed gallery forest (dgf) × undisturbed savanna (sv); (2) undisturbed gallery forest × disturbed gallery forest; (3) undisturbed gallery forest × urban environment (ur); (4) undisturbed savanna × urban environment

Comparison	Species	IndVal (%)		
1		gf	dgf	sv
	D. hydei	2	20	61
2		gf	dgf	
	D. hydei	4	52	
3		gf	ur	
	D. busckii	9	79	
	D. mercatorum	23	77	
	D. prosaltans	22	73	
	D. simulans	25	75	
	S. latifasciaeformis	7	83	
	Z. indianus	2	98	
4		sv	ur	
	D. busckii	33	57	
	D. immigrans	4	88	
	D. prosaltans	13	83	
	D. simulans	11	89	

Comparison	Species	IndVal (%)	
	S. latifasciaeformis	30	59
	Z. indianus	43	57

Two-stage indicator validation

Although several species were identified initially as characteristic species of undisturbed gallery forests and savannas, only five were re-identified as characteristics species when testing using the two approaches (independent data set and random data subsampling; Table 5). Drosophila ornatifrons, D. paraguayensis, and D. willistoni emerged as robust characteristic species of gallery forests, and D. mercatorum and Z. indianus of savanna environments (Table 5).

Table 5
Species identified as characteristic of undisturbed gallery forests and undisturbed savannas, in the two-stage identification process for RECOR I/NP and RECOR II data sets

Approach	Data set	Habitat types	
Independent sample		Undisturbed gallery forest (n = 2)	Savanna (n = 2)
	RECOR I/NP (identification)	D. bocainensis	D. busckii
		D. fuscolineata	D. nebulosa
		D. guaru	S. laficasciaeformis
		D. maculifrons	
	RECOR II (validation)	(n = 3)	(n = 6)
		D. mediostriata	D. cardini*
		D. prosaltans	D. nigricruria*
			D. polymorpha*
			D. simulans
	RECOR I/NP and RECOR II	D. immigrans*	D. hydei*
		D. ornatifrons*	D. mercatorum*
		D. paraguayensis*	Z. indianus*
		D. willistoni*	
Random selection		Undisturbed gallery forest (n = 3)	Savanna (n = 4)
	Selection I (identification)	D. immigrans	
		D. maculifrons	
		D. malerkotliana	
		D. mediopunctata*	
		D. mediostriata	
		D. polymorpha	
		D. schildi*	

Approach	Data set	Habitat types	
		D. simulans	
		D. sturtevanti	
	Selection II (validation)	(n = 2)	(n = 4)
		D. prosaltans	D. busckii
			D. cardini
			D. hydei
			D. malerkotliana
			D. nebulosa
			D. nigricruria
			D. simulans
			D. sturtevanti
			S. latifasciaeformis
	Selection I and II	D. ornatifrons	D. mercatorum
		D. paraguayensis*	Z. indianus
		D. willistoni	

* P < 0.05

The underlined species were re-identified as characteristics species when testing using the two approaches (independent data set and random data subsampling), and therefore, they emerged as robust characteristic species

Discussion

The present survey has formally tested the potential indicator of drosophilids in the Cerrado biome, by following up a methodology that associates the Indicator Value method (Dufrene and Legendre *1997*) and the two-stage indicator validation (McGeoch et al. *2002*). A set of reliable indicator variables were found at both assemblage and species levels, that together, are likely to be efficient in reflecting a variety of ecological conditions. The undisturbed forests supported the greatest number and dominance of neotropical and narrow range species, while the exotic and widespread species dominated the disturbed forests, savannas, and urban environment. Therefore, evaluating changes in the raw and relative abundances, as well as in the richness of different species categories, provides a good overview of the alterations in drosophilid assemblages in different habitats. This kind of information can be useful not only in diagnostic surveys, but also in monitoring the incidence of disturbance over time.

At the species level, most of species that showed specificity for undisturbed forests did not reach the status of indicator because the fidelity component of the IndVal was not sufficiently high (Dufrene and Legendre *1997*). Although these species demonstrated preference for undisturbed forests, many of them being even exclusive from these habitats, they were only found in one or few sites, and in very low densities, which means that it will be extremely difficult and improbable to find these species in other sites or investigations (McGeoch and Chown *1998*). On the other hand, five reliable indicators emerged from the identification and testing process for undisturbed gallery forests (Drosophila paraguayensis, D. ornatifrons, and D. willistoni) and savannas (D. mercatorum and Zaprionus indianus) as they showed high frequency of occurrence (fidelity), and preference (specificity) for these habitats across independent data sets (see also Tidon *2006*). Saavedra et al. (*1995*) have already suggested D. willistoni as an indicator of conserved Atlantic Forest environments. Therefore, due to their high reliability and specificity to particular habitats,

such characteristic species are very useful in diagnostic surveys. D. cardinoides and D. melanogaster emerged as indicators of urban environments; however, they could not be validated due to the absence of independent data sets for these environments.

Because Drosophila hydei was more prevalent in intermediate habitat (disturbed gallery forests) than in the less preferred habitat stage (undisturbed forest), but still had a moderate preference for the other habitat stage (savanna), it was the only species that matched the original prediction for detector species (McGeoch et al. *2002*). Besides this one, other seven species also increased their preferences for disturbed environments, when compared to the undisturbed stage, but they did not have an intermediate preference for savannas as expected by the original prediction. Nonetheless, these species clearly demonstrated a change in habitat between the two stages (from undisturbed to disturbed forest), and for this reason, they were also regarded as detector species. Detector species are a useful class of ecological bioindicator, since they are indicative not only of the changes in habitat conditions, but also of the direction of such changes, and therefore, they are very useful in monitoring surveys. Characteristic and detector species provide complementary information with higher information content and reliability than either group on this own (McGeoch et al. *2002*). The use of a group of indicator variables, which are complementary, is considered critical and indispensable for bioindication studies. This approach will minimize the dependence on an individual taxon and improve the system reliability, since the conclusions will be based on a greater variety of measures (Hilty and Merenlender *2000*).

The present results corroborate that the IndVal method (Dufrene and Legendre *1997*) associated with two-stage indicator validation correspond to an excellent tool for developing bioindicators. Besides all of the advantages previously discussed, the IndVal has also supplied essential information on drosophilid species preferences, subdividing them into different assemblages, typical to the habitat types and presence of disturbance. The sensitivity of the method in classifying the assemblages was very useful considering the large heterogeneity of habitats in the Cerrado. Describing how the assemblages are organized, and at which scale, is a fundamental step for understanding the mechanisms involved on their regulation (Dufrene and Legendre *1997*). Moreover, the IndVal method takes into account the identity of each species, and therefore produces qualitative rather than the quantitative results produced by other diversity indexes. Such qualitative approach is extremely suitable for studies related to biodiversity conservation.

McGeoch et al. (*2002*) have advocated that the two-stage indicator validation is essential for all studies concerned with indicator development and the present study confirm this recommendation. A large number of species were identified as characteristic of a particular habitat, but only those that were validated by the two approaches were recognized as reliable indicators. Species that were initially identified as indicator but were not validated were then considered as unreliable. By using this method, the degree of reliability of the final set of identified indicators was greatly improved and refined. It is highly recommended that future studies validate the other findings of this study, such as the characteristic species of urban environments and the detector species.

Drosophilids as indicators of human change on the Cerrado habitats

The potential of drosophilid species as bioindicators was formally tested and confirmed. A set of criteria for the selection of ecological indicators has been established in literature in the last 20 years (Landres et al. *1988*; Noss *1990*; McGeoch *1998*; Caro and O'Doherty *1999*; Hilty and Merenlender *2000*; Dale and Beyeler *2001*; Niemi and McDonald *2004*), and in general, drosophilids fulfil many of these criteria. These organisms are regarded as excellent biological models for ecological research because they are small, numerous, easily collected and manipulated, and relatively cheap to maintain (Powell *1997*; Brookes *2001*). Consequently, they have been broadly studied, and there is a large amount of reliable and available

information about them (Bächli *2007*; Grumbling and Strelets *2007*). Currently, this family includes more than 3,500 described species (Bächli *2007*). Some species, that exploit a very broad range of plants, evolved as human commensals, spreading around the world. Other species exploit a very narrow range of breeding sites, being extremely specialized and restricted to a single type of environment (Powell *1997*). As a result, they are widespread around the world, being found in all biogeographical regions, and occurring in a variety of habitat types, from pristine to urban environments (Wheeler *1981*, *1986*). Many species are also closely associated to environmental variables, being extremely sensitive to changes on the habitats conditions (Karan et al. *1998*; Jenkins and Hoffmann *2001*; Van Klinken and Walter *2001*; Avondet et al. *2003*; Hoffmann et al. *2003*). These features associated to a short life cycle allow these organisms to be anticipatory, as well as to be used in monitoring changes in habitats conditions. Moreover, associations between drosophilids and the Cerrado habitats have already been suggested. Even though the family is widely distributed in the biome, the assemblages vary substantially in abundance and composition between forests and savannas, and across seasons (Tidon *2006*); along an urban gradient (Ferreira and Tidon *2005*); and also reflecting the effects of human disturbances at a local scale (Mata *2002*).

The present evaluation of the drosophilid species in Cerrado suggests that the undisturbed gallery forests are extremely special habitats in the Cerrado biome because they were the richest studied habitat, and were also most unique, given the predominance of rare species. Rare species are often the focus of conservation strategies due to their higher vulnerability to extinction (IUCN *2007*). In contrast, abundant and widespread species that showed low preference for undisturbed forests were more abundant in the disturbed stage. The majority of species of several taxa also demonstrates preference or is associated with gallery forests, for instance, plants (Mendonça et al. *1998*), mammals (Redford and Fonseca *1986*), birds (Silva *1995*), butterflies (Brown *2000*), and wasps (Diniz and Kitayama *1998*). Therefore, although gallery forests occupy <10% of all Cerrado extension, these habitats are extremely important to Cerrado biodiversity (Oliveira and Marquis *2002*). However, these habitats have intensively been substituted by human environments, what causes habitat loss and fragmentation (Silva et al. *2006*), and once transformed, regeneration to an original condition is extremely improbable (Pivello and Coutinho *1996*). If such disturbances persist for a long time, they will probably cause an irreversible impoverishment in the regional biota of this rich ecosystem. For instance, the original different drosophilid assemblages from forests and savannas can become more similar, since abundant and widespread species that dominate the savannas invade gallery forests when they are disturbed.

In conclusion, the results presented here provided information that can be extremely helpful to develop and improve management for the complex forest–savanna mosaic present in the Cerrado biome. In addition, the bioindicator system proposed represents a novel option, complementing the still few surrogate taxa used in this biome, generally plants and vertebrates, that alone, provide only a limited view of the environment. These indicators may be very helpful to reflect disturbance incidence at the beginning, in monitoring and restoring conservation programs and to refine the selection, planning, and management of the reserve areas. Nevertheless, it can help in clarifying mechanisms by which human activities affect biodiversity and facilitate improved projections about what might happen in the future. Studies intending to test this system, and to validate some of the results found here by incorporating other habitat types, using standardized methodology, independent samplings (at spatial and temporal scales), as well as measuring environmental variables, are essential to continuously improve its reliability and robustness.

Appendix 1

Abundance of drosophilid species identified in this study and number of sites in which they occurred

Species	Abundance	Sites
Zaprionus indianus [a] Gupta, 1970	32,110	19
Drosophila simulans [a] Sturtevant, 1919	19,900	19
D. willistoni Sturtevant, 1916	10,574	16
D. sturtevanti Duda, 1927	4,402	19
D. mercatorum Patterson & Wheeler, 1942	2,885	19
D. nebulosa Sturtevant, 1916	1,719	19
D. cardini Sturtevant, 1916	1,276	19
D. malerkotliana [a] Parshad & Paika, 1964	1,173	16
D. polymorpha Dobzanhsky & Pavan, 1943	807	16
D. immigrans [a] Sturtevant, 1921	680	14
Scaptodrosophila latifasciaeformis [a] Duda, 1940	436	12
D. paraguayensis Duda, 1927	278	5
D. ornatifrons Duda, 1927	213	12
D. hydei Sturtevant, 1921	167	12
D. cardinoides Dobzanhsky & Pavan, 1943	142	7
D. nigricruria Patterson & Mainland, 1943	90	13
D. prosaltans Duda, 1927	80	16
D. busckii [a] Coquillet, 1901	77	11
D. maculifrons Duda, 1927	50	7

Species	Abundance	Sites
D. fuscolineata Duda, 1925	33	6
D. mediostriata Duda, 1925	31	11
D. mediopunctata Dobzhansky & Pavan, 1943	27	5
D. austrosaltans Spassky, 1957	22	3
D. neocardini Streisinger, 1946	20	3
D. aragua Vilela & Pereira, 1982	16	7
D. paranaensis Barros, 1950	15	5
D. atrata Burla & Pavan, 1953	10	2
D. bocainensis Pavan & Cunha, 1947	9	4
D. schildi Malloch, 1924	7	4
D. mesostigma Frota-Pessoa, *1954*	7	1
D. guaru Dobzanhsky & Pavan, 1943	6	4
D. pallidipennis Dobzanhsky & Pavan, 1943	5	2
D. melanogaster [a] Meigen, 1830	4	3
D. bandeirantorum Dobzhansky & Pavan, 1943	4	2
D. onca Dobzanhsky & Pavan, 1943	3	1
D. ararama Pavan & Cunha, 1947	2	2
D. arauna Pavan & Nacrur, 1950	2	1
D. fumipennis Duda, 1925	1	1
D. medioimpressa Frota-Pessoa, *1954*	1	1
D. neoguaramunu Frydenberg, 1956	1	1

Species	Abundance	Sites
D. paramediostriata Townsend & Wheeler, 1955	1	1
Total	77,286	19

[a]Exotic species

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