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ENVIRONMENTAL STRESS AND FLUCTUATING ASYMMETRY IN *Antilophia galeata, Myiothlypis flaveola* AND *Basileuterus culicivorus* IN BRAZILIAN SAVANNA

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Abstract: Fluctuating asymmetry (FA), defined as the random difference between two sides of a bilaterally symmetrical character, is often used to monitor biological populations in altered habitats. We aimed to compare the values of FA for wing and tarsi of three bird species (*Antilophia galeata*, *Myiothlypis flaveola* and *Basileuterus culicivorus*) in areas with different environmental stresses and to analyze their potential use as biomonitors. The birds were captured between March 2010 and March 2011, in seven forest fragments. In areas of high environmental stress, FA was higher for the wings of *A. galeata* and *M. flaveola* and the tarsi of *B. culicivorus*. FA depends on the functional importance of the character for each species. Thus, this study demonstrated that FA in wings and tarsi is a useful tool to assess the quality of the Brazilian Savanna (Cerrado) forest habitat.

Key-words: anthropogenic disturbance; biomonitors; environmental quality.

INTRODUCTION

Fluctuating asymmetry (FA) is a phenotypic manifested as the variable left–right difference in size or shape of bilaterally symmetric structures or as the variation among repeated parts in structures with complex symmetry (Palmer & Strobeck 2003, Van Dongen 2006, Klingenberg 2015, Grebemichael *et al.* 2019). FA is widely used in ecology and evolutionary biology as an easily measurable indicator of environmental and genetic stress (Anciães & Marini 2000, Beasley *et al.* 2013, Abeli *et al.* 2016, Coda *et al.* 2017, Grebemichael *et al.* 2019), despite it may reflects the individual's intrinsic ability to react to a range of biotic, abiotic and genetic stressors (Trokovic *et al.* 2012, Coda *et al.* 2017). It is a practical and reliable tool for environmental monitoring (Clarke 1992, Vangestel & Lens 2011) since the data allow a quantitative assessment of stress to which populations are subjected (Anciães & Marini 2000, Mamedova 2009). Beasley *et al.* (2013) conducted a meta-analysis of published literature to test the hypothesis that FA is a reliable biomarker of environmental stress in insects and concluded the use of FA as a biomarker of environmental stress is a legitimate tool particularly when studies verify the biological relevance of stressors for the study organism.

FA in birds is useful for biomonitoring natural and modified environments (Bonisoli-Alquati & Mousseau 2013, Grebemichael *et al.* 2019). For example, in Passeriformes of the Atlantic Forest, wing and tarsus FA were significantly greater in fragments than continuous areas for the whole community and were both negatively correlated with forest fragment size. (Anciães & Marini 2000). Besides *et al.* (2017) showed that FA increased with mercury concentrations in whole blood and breast feathers of *Sterna forsteri* (Charadriiformes: Laridae) a species with elevated mercury concentrations, in San Francisco Bay, California, USA. Furthermore, FA can affect other aspects of species' biology, such as sexual selection. Variations in the tail feathers of *Hirundo rustica* (Passeriformes: Hirundinidae) indicated that there is a negative correlation between FA and mating success (Rasmuson 2002, Grebemichael *et al.* 2019).

We aimed to compare the values of FA for wing and tarsi of *Antilophia galeata* (Passeriformes: Pipridae), *Myiothlypis flaveola* (Passeriformes: Parulidae) and *Basileuterus culicivorus* (Passeriformes: Parulidae) between areas under different environmental stresses, considering that higher levels of FA strongly related with high environmental stresses. The three bird species used in this study are habitat specialists, dependent on forests (Manhães & Loures-Ribeiro 2011) and commonly recorded in Brazilian Savanna (Malacco *et al.* 2013). This dependency makes them more susceptible to environmental stress because they rely on specific disturbance-sensitive habitat conditions, such as the floristic and structural vegetation patterns (Lee & Peres 2008, Martensen *et al.* 2008). Also, if their forest environments are under stress, these species are unlikely to move across open areas (Anciães & Marini 2000, Hansbauer *et al.* 2008).

MATERIAL AND METHODS

Study areas

We conducted the study in seven fragments of seasonal semideciduous forest located in the Brazilian Savanna in the state of Minas Gerais (Figure 1). The Köppen-Geiger climate classification is AW and the average annual temperature is 21.5 °C (70.7 °F) (Alvares *et al.* 2013). The fragments were under different levels of environmental stress. We determined the level of environmental disturbance using a protocol for rapid assessment of the quality of terrestrial environments, adapted from Rodrigues & Castro (2008). This protocol included nine parameters: the occupation of the surrounding matrix, presence of tracks, disposal of solid waste in the area, presence of exotic flora and cattle inside the forest, tourism, proximity to the urban area, outside noise and evidence of recent fires. The parameters were scored with the values: 0, 1, 3 and 5: being 0 of the lowest level of stress and 5 the highest level of environmental stress. We classified each area with environmental stress level as low (scores 1 to 10), medium (scores 11 to 20) and high (scores 21 to 30) (Table 1).

Data collection

We captured birds between March 2010 and March 2011 using minimum of 12 and maximum of 25 mist nets (12 x 3 m; mesh: 30 mm, nylon). We checked the mist nest every 30 minutes. We identified bird species using specialized guides and we followed the nomenclature based on Brazilian Committee for Ornithological Records (CBRO) (Piacentini *et al.* 2015). Each bird was marked with metal rings (CEMAVE/ICMBio - Authorization: 2943 and 3238). For the analyzes, we only considered full-grown adult birds (CEMAVE 2014) and species that occurred in all fragments and with minimum of five individuals. Thus, *A. galeata*, *M. flaveola* and *B. culicivorus* are used.

For each bird captured, we measured the lengths of the left and right wings and tarsi three times (CEMAVE 1994) with a digital caliper (0.01 cm). We took the measurements from each side alternately to avoid sampling bias and released the animals immediately (Roos 2010). We calculated the arithmetic means for each character per species.

Fluctuating asymmetry estimate

We evaluated the FA separately for the wing and tarsi of each species, following Palmer & Strobeck (1986) and Anciães & Marini (2000), as: $FA = \Sigma|(R - L)|*N^{-1}$, where R and L are the arithmetic means of the right and left side of the structure,

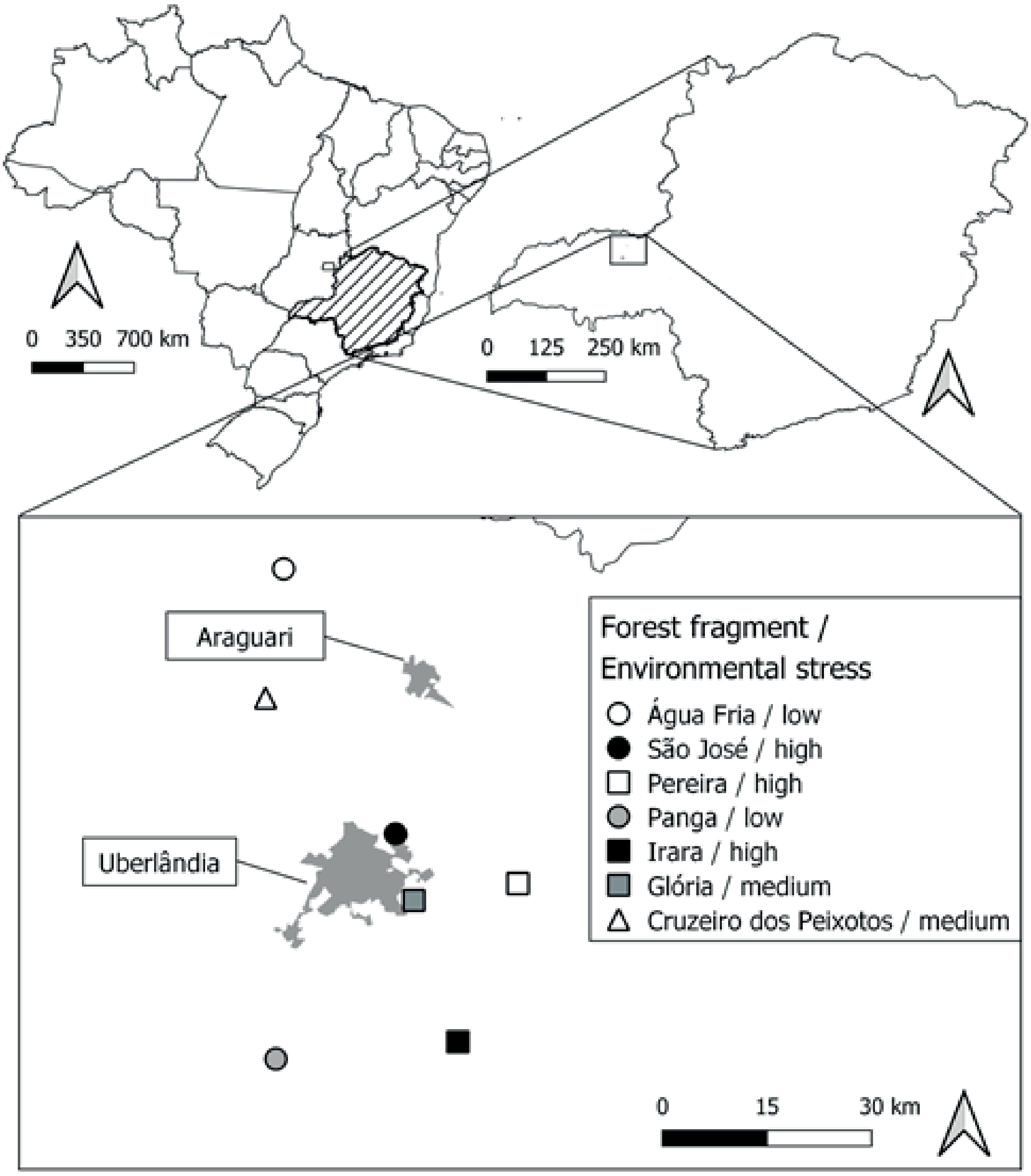

Figure 1. Map indicating the location of forest fragments, located at Brazilian Savanna in Minas Gerais state. Author: Vitor Carneiro de Magalhães Tolentino.

respectively; and N is the number of individuals in the sampling. We distinguish FA from other types of asymmetries, such as directional asymmetry and antisymmetry, by statistical tests (Palmer & Strobeck 1986). Directional asymmetry is a type of asymmetry with a clear predominance of either right or left structures and antisymmetry is a kind of bilateral asymmetry, characterized by low kurtosis values of difference between left and right side values (Palmer & Strobeck 1986, Baranov 2018).

Statistical analysis

We tested the difference in FA for the characters of each species in different areas using one-way analysis of variance (Zar 2010). We conducted the analyses in Systat 10.2 software. The data showed normal distribution with averages equal to zero for wings and tarsi and the measurement errors were considered insignificants ($p < 0.001$), according to basic premises suggested by Anciães & Marini (2000).

RESULTS

We captured 59 individuals of *A. galeata* (low: 8, medium 22; high: 29), 30 of *M. flaveola* (low: 11, medium: 3, high: 17) and 26 of *B. culicivorus* (low:

Table 1. Name, coordinates and environmental stress level of the seven fragments of seasonal semideciduous forest analysed, located at Brazilian Savanna in Minas Gerais state.

Forest fragment	Geographic coordinates	Environmental stress (score)
Água Fria	18°29’S; 48°23’W	Low (4)
Panga	19°10’S; 48°23’W	Low (4)
Cruzeiro dos Peixotos	18°40’S; 48°24’W	Medium (14)
Glória	18º57’S; 48º12’W	Medium (13)
Irara	19°08’S; 48°08’W	High (21)
Pereira	18°55’S; 48°03’W	High (21)
São José	18°51’S; 48°13’W	High (30)

8, medium: 10; high: 8). For these individuals, antisymmetry and directional asymmetry, respectively, were discarded; thus, they presented FA for wings (Table 2) and tarsi (Table 3).

A. galeata and *M. flaveola* exhibited higher FA for the wings in areas with high environmental stress ($F_{2,56} = 7.347$, $p = 0.001$ / $F_{2,27} = 3.776$, $p = 0.036$, respectively); likewise, the tarsi of *B. culicivorus* were more asymmetric in these areas ($F_{2,23} = 1.169$; $p = 0.032$) (Figure 2).

DISCUSSION

The intense use of a character (i.e. wings or tarsi) during the life of a bird, in activities such as flight and foraging, can influence the FA, despite efficiency in character stabilization can be reduced under stressful conditions (Balmford *et al.* 1993, Anciães & Marini 2000, Vangestel & Lens 2011, Trokovic *et al.* 2012, Coda *et al.* 2017, Grebemichael *et al.* 2019). Therefore, although any bilateral symmetrical structure can present FA, in environmental assessment it only makes sense to use those characters that affect individual fitness (Almeida 2003, Gonçalves & Melo 2012). FA has been the most commonly used index to describe phenotypic variations caused by environmental stress (Gonçalves & Melo 2012, Herring, Collin & Ackerman 2017).

Antilophia galeata consumes fruits mainly during flight (Marini 1992, Silva & Melo 2011, Pires & Melo 2019). Flight maneuvers often wear out the wings by putting them in more contact with the environment, suffering the influence of stressors that makes it difficult to stabilize the character (Gonçalves & Melo 2012). Thus, the FA for wings of *A. galeata* will likely be higher in disturbed areas because individuals will spend more time foraging and increase wings’ exposure. Therefore, it can be an efficient biomonitor to assess environmental quality by comparing the morphological data from different sites.

As for *A. galeata*, *M. flaveola* also showed high values of FA for wings in disturbed habitats. This species is a flying forager who chase winged termites on forest edges and vertically inside the forest (Marini & Cavalcanti 1993, Boesing, Nichols & Metzger 2018, Curson 2019, de Godoy & Gabriel 2019), thus wearing out the wings. The microhabitat in the forest edges and disturbed areas with open canopies have more light incidence, higher temperatures and stronger winds (Pfeifer *et al.* 2017). We believe that these disturbances can influence wings’ FA, because the wings are keratinized, and all disturbances above-mentioned can wear out them. Despite *M.*

Table 2. Fluctuating asymmetry in wing (mean ± standard deviation, centimeters) for three species from habitats with different level of environmental stress. In parentheses, the number of samples.

Species	Environmental stress		
	Low	Medium	High
Antilophia galeata (59)	0,003 ± 0,003 (8)	0,002 ± 0,002 (22)	0,004 ± 0,004 (29)
Myiothlypis flaveola (30)	0,003 ± 0,002 (11)	0,002 ± 0,002 (3)	0,008 ± 0,006 (17)
Basileuterus culicivorus (26)	0,002 ± 0,002 (8)	0,003 ± 0,002 (10)	0,001 ± 0,001 (8)

Table 3. Fluctuation asymmetry in tarsi (mean ± standard deviation, centimeters) for three species from habitats with different level of environmental stress in Brazilian Savanna, in Minas Gerais state. In parentheses, the number of samples.

Species	Environmental stress		
	Low	Medium	High
Antilophia galeata (59)	0,005 ± 0,006 (8)	0,002 ± 0,001 (22)	0,007 ± 0,012 (29)
Myiothlypis flaveola (30)	0,004 ± 0,005 (11)	0,003 ± 0,002 (3)	0,005 ± 0,005 (17)
Basileuterus culicivorus (26)	0,002± 0,001 (8)	0,003 ± 0,002 (10)	0,012 ± 0,021(8)

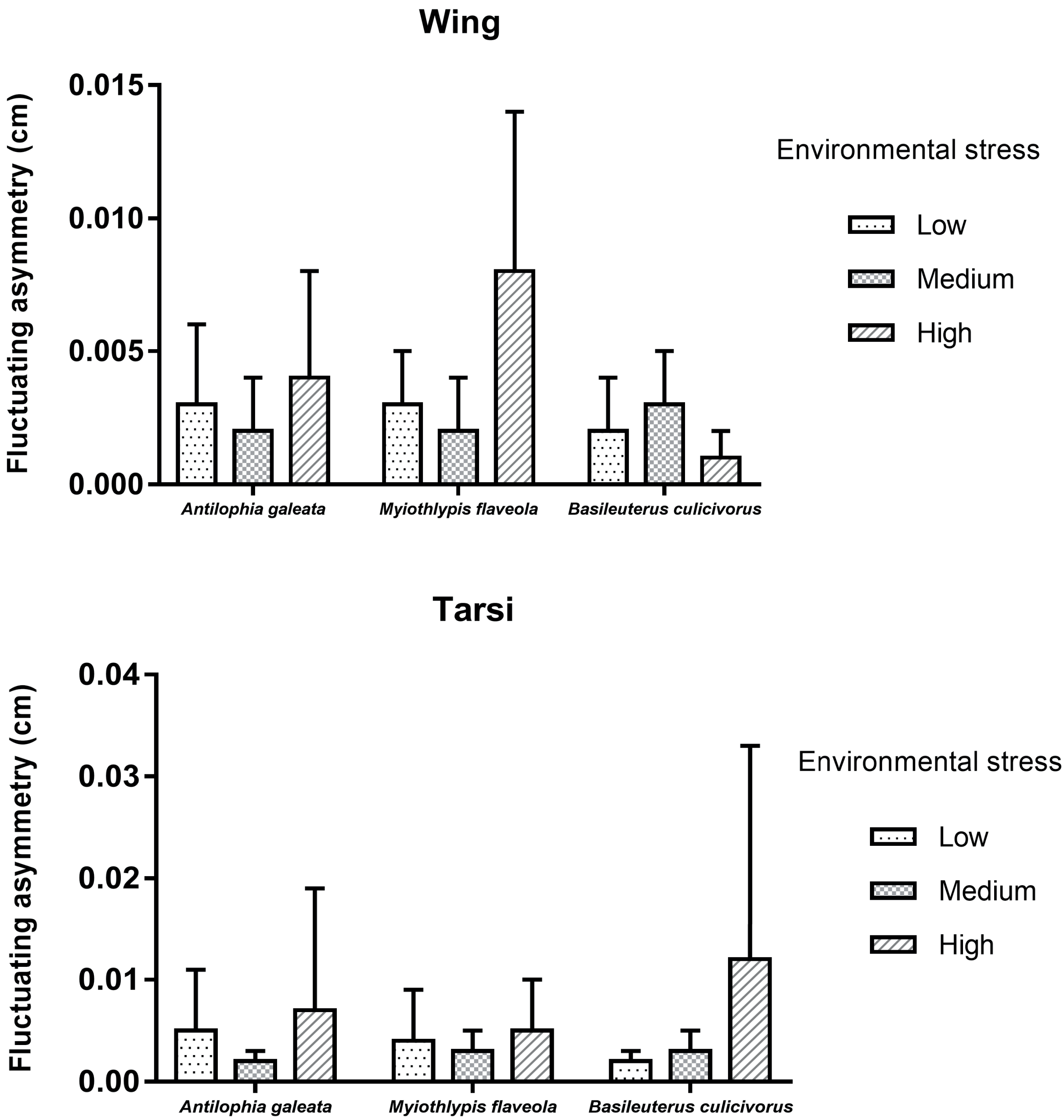

Figure 2. Comparison among the Fluctuation asymmetry in wing and tarsi for three species from different level of environmental stress in Brazilian Savanna, in Minas Gerais state.

flaveola showed high values of FA, the low samples size may the test less robust. So, it is important, in future studies, that the sample is higher.

On the other hand, *B. culicivorus* had higher values of FA only for the tarsi. This species uses tarsi to predate insects in the forest's understory, including ground-foraging (Marini & Cavalcanti 1993). Moreover, it moves jumping on vertical branches, vines and roots (Sigrist 2006). Thus, we suggest that the frequent use of the tarsi may put them under constant stress and that they will be more asymmetric in higher levels of environmental disturbances (Lens *et al.* 2002a,b). FA reflects the ability of an organism to develop precise characters due to genetic or environmental alterations (Almeida 2003).

Wings are flexible structures and wear out more easily in contact with the air. Rigid structures, such as the tarsi and other bones, can reduce the measurement bias and provide more accurate measures of FA (Hutchison & Cheverud 1995, Almeida 2003). Thus, we suggest that the tarsi should be a standard structure to evaluate FA, but that wings can be used in those species that use them more frequently during foraging. However, it is important to consider the development of characters throughout the life history of individuals, since conservation practices that consider only the current abundance and movements may be inadequate (Coster *et al.* 2013). We also urge researchers to avoid measuring worn and molting feathers, because they can affect FA values.

Our findings suggest that the wings of *A. galeata*, *M. flaveola* and the tarsi of *B. culicivorus* may be used for biomonitoring the Brazilian Savanna forest environments, because wing or tarsus FA were significantly greater in fragments than has highest level of environmental stress.

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