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Original article

Wing symmetry in wild drosophilids (Insecta, Diptera) is not affected by season in the Brazilian Cerrado.

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ABSTRACT: The development of an organism is a controlled process, which can be disrupted by genetic or environmental stress. Although fluctuating asymmetry is widely used as an indicator of developmental instability, its effectiveness has been questioned due to the contradictory results produced by this technique which, at least in part, probably reflects methodological inappropriateness. Here, we investigated if wing asymmetry of drosophilids increases when they develop during the dry season in the Brazilian savanna, considered a stressful season for these insects. Using protocols designed to avoid methodological problems, we analysed the wings of *Zaprionus indianus* and three species of the genus *Drosophila* (*D. mercatorum*, *D. simulans*, and *D. sturtevanti*). There was no significative difference in wing asymmetry in any of the four species between the dry and rainy seasons. The similar wing asymmetry levels between seasons may mean that during the dry season drosophilids are submitted to strong natural selection and the asymmetric individuals have less chance of surviving. Alternatively, environmental drought may not affect the wing symmetry. Although our study added more data to the relationship between asymmetry and stress, this discussion seems to be far from being solved.
Key words: Brazil, environmental stress, fluctuating asymmetry, seasonal variation.

RESUMO (A assimetria de drosofilídeos (Insecta, Diptera) não é afetada pelas estações no Cerrado brasileiro): O desenvolvimento de um organismo é um processo controlado, que pode ser alterado por estresses genéticos ou ambientais. Embora a assimetria flutuante seja amplamente utilizada como um indicador de instabilidade do desenvolvimento, sua eficácia tem sido questionada devido aos resultados contraditórios produzidos por essa técnica que, pelo menos em parte, provavelmente reflete inadequação metodológica. Aqui, nós investigamos se a assimetria das asas dos drosofilídeos na savana brasileira aumenta durante estação seca, considerada estressante para esses insetos. Usando protocolos elaborados para evitar problemas metodológicos, analisamos as asas de *Zaprionus indianus* e três espécies do gênero *Drosophila* (*D. mercatorum*, *D. simulans* e *D. sturtevanti*). Não foram encontradas diferenças significativas na assimetria das asas de nenhuma das quatro espécies entre as estações seca e chuvosa. Os níveis semelhantes de assimetria nas duas estações podem significar que durante a estação seca os drosofilídeos são submetidos a forte seleção natural e os indivíduos assimétricos têm menos chance de sobreviver. Alternativamente, a baixa umidade ambiental pode simplesmente não afetar a simetria das asas. Embora nosso estudo tenha adicionado mais dados à relação entre assimetria e estresse, essa discussão parece estar longe de ser resolvida.
Palavras-chave: Brasil, estresse ambiental, assimetria flutuante, variação sazonal.

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INTRODUCTION

Developmental instability can be described as the predisposition of a developmental system to produce a morphological change in response to random perturbations (Dongen 2006, Klingenberg 2019). Environmental stresses, such as extreme temperatures or shortage of resources, can disrupt the developmental process, increasing developmental instability and altering the morphology of the adult (Markow 1995, Moller & Swaddle 1997). This happens because, under optimal conditions, the developmental process follows a genetically determined pathway. Since under stressful conditions the efficiency of the stability mechanisms may be reduced, the organism is not able to maintain its original pathway, producing unexpected phenotypes (Clarke 1995). Therefore, indicators of developmental instability can be useful tools to identify stress before they cause detrimental impacts on populations.

Fluctuating asymmetry (FA) has been widely used as a method to measure developmental instability (Moller & Swaddle 1997, Benítez & Parra 2011, Beasley *et al*. 2013, Klingenberg 2015). It is one of the three patterns of asymmetry, each characterized by a different combination of mean and variance of the distribution of right minus left (R – L) differences (Palmer & Strobeck 1986). Directional asymmetry is characterized by a normal distribution of (R–L) with the mean different from zero, antisymmetry for a bimodal distribution of (R–L) with the mean zero, and FA for a normal distribution of (R–L) with the mean zero. Briefly, the underlying assumption of FA analysis is that the development on both sides of a bilateral organism occurs in the same environment and that the same genes and processes control it. Therefore, any differences between sides must be the result of errors during development (Clarke 1995, Hosken *et al*. 2000).

Several studies have found detrimental effects of environmental stresses in the symmetry of organisms. For example, the amount of FA of tropical birds was negatively correlated with the size of the forest fragment they occupied (Anciães & Marini 2000). Likewise, specimens of *Drosophila melanogaster* developed under larval overpopulation were more asymmetrical than those developed under low density (Imasheva & Bubliy 2003). Neotropical tadpoles subjected to disturbed habitats were more asymmetric than those established in preserved environments (Costa *et al*. 2017), and salmons from hatchery-origin were more asymmetric than wild salmon (Koeberle *et al*. 2020).

Other studies, however, did not detect any effect of environmental stress on the FA. Ambo-Rappe *et al*. (2008) found no correlation between heavy metal contamination and FA measures of the seagrass *Halophila ovalis* in a lake submitted to different levels of pollution. Similarly, the stressful effects of thermal shock and dissection in *Cyrtodiopsis dalmanni* had no reflection on asymmetry (Bjorksten *et al*. 2001), and house sparrows exposed to variable levels of nutritional stress did not show different levels of FA in tarsus or rectrix length (Vangestel & Lens 2011). These frequent disagreements among studies have questioned the efficiency of FA as an index of developmental instability. Issues such as the choice of the study organism and the traits evaluated, statistical problems, absence of measurement error, and few replications, are highlighted as possible sources of mistakes in the studies (Lens *et al*. 2002, Coster *et al*. 2013, Pertoldi & Kristensen 2015).

Drosophilid wings are particularly appropriate for studying asymmetry because they are basically bidimensional organs presenting a relatively simple morphology. Additionally, these flies are small, short-lived, easily collected and manipulated, generate numerous offspring, and are sensitive to variations in environmental conditions (Powell 1997). Because of these advantages, some species of the family Drosophilidae are widely used as biological models (Mata *et al*. 2010a, Mohr 2018).

The *Cerrado* biome, also known as the Brazilian savanna, is the second largest biome of South America in extension and one of the richest savannas of the world. It is characterized by two well-defined seasons (Ribeiro & Walter 1998): a dry season, usually from May to September, and a rainy season, usually from November to March (Figure 1). Because most of its drosophilid species suffer an accentuated populational reduction during the dry season (Tidon 2006), we presume this season is stressful for drosophilids, as is the case for other insects (Wolda 1988, Pinheiro *et al*. 2002).

Here, we investigated if fluctuating asymmetry in four drosophilid species reflect ontogenetic disturbances caused by natural environmental stress. We hypothesized that, in the Brazilian savanna, drosophilids developed in the dry season would be more asymmetrical than those developed in the rainy season.

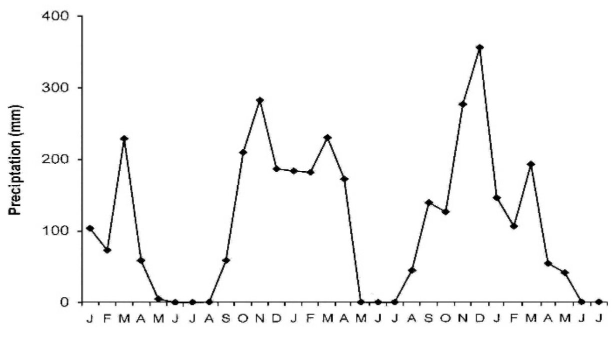

Figure 1. Monthly variation of precipitation in the area from January 1999 to July 2001 (www.recor.org.br). Months of the year represented by their first letters.

MATERIALS AND METHODS

The typical vegetation landscape of the *Cerrado* biome consists of a savanna of very variable structure on the well-drained interfluves, with gallery forests or other moist vegetations following the watercourses (Oliveira & Marquis 2002). The flies sampled in this study were collected in savanna-like vegetation, locally known as *cerrado sensu stricto*, characterized by a predominantly grassy ground layer with scattered herbs, and a woody layer of trees and shrubs, with thick, corky, bark, and coriaceous leaves (Ribeiro & Walter 1998). Among the 133 drosophilid species recorded in this biome (Roque *et al*. 2016), we evaluated the two most abundant and widely distributed neotropical (*Drosophila mercatorum* and *D. sturtevanti*) and exotic (*D. simulans* and *Zaprionus indianus*) species in the region.

Drosophila mercatorum Patterson and Wheeler belongs to the *repleta* group of the subgenus *Drosophila* and includes two subspecies. *Drosophila mercatorum mercatorum* occurs in Peru, Colombia, Central America, North America, Australia, Africa, Europe, and Asia, and it is usually associated to man. *Drosophila mercatorum pararepleta*, studied here, is distributed to the east of Andes, in open vegetations, dry areas, and forests (Manfrin *et al*. 1997). The other neotropical species, *Drosophila sturtevanti* Duda, belongs to the *saltans* group of the subgenus *Sophophora* and is also widely distributed in the Neotropical Region, although it is more abundant in areas of open vegetation (Sene *et al*. 1980).

Drosophila simulans Sturtevant belongs to the *melanogaster* group of the subgenus *Sophophora*. It is the most widely distributed exotic species in the Neotropical region and also the most abundant in several collections performed across South America (Sene *et al*. 1980, Tidon-Sklorz & Sene 1999). Finally, *Zaprionus indianus* Gupta was introduced into South

America probably at the end of 1998 (Vilela 1999), and since then quickly dispersed throughout the Neotropical region (Tidon *et al*. 2003, Galego & Carareto 2010). This last species is a successful colonizer that has adapted to different climatic conditions (Mata *et al*. 2010b).

The drosophilids sampled in this study were collected in the Ecological Reserve of IBGE (RECOR) near Brasília, capital of Brazil (15º56'S; 47º53'W). Supplementary information about this area and methods of collection have been previously published in Tidon (2006). *Drosophila mercatorum* and *D. simulans* were collected in June, July and August of 1999 and January of 2000, while *D. sturtevanti* and *Zaprionus indianus* were collected in January of 2000 and in June and July of 2001. The flies were initially identified using the identification-key of Freire-Maia & Pavan (1949), and the taxonomic determination of *Drosophila mercatorum* and *D. simulans* was confirmed by the analysis of the male terminalia (Vilela 1983) and anal plates, respectively. For this reason, only the males of these two species were used in the analyses. Samples sizes of the measured flies are shown in Table 1.

The two wings of each fly were dissected, identified (right and left) and mounted on a slide. A microscope connected to a computer was used to capture wing images. The wing parameters evaluated followed Klaczko & Bitner-Mathé (1990), which put down an ellipse from Cartesian co-ordinates taken from 40 points on the wing outline (Figure 2). From the mathematical relationships between the radii "a" and "b" of the ellipse it is possible to obtain a size measure free from shape (SI = √ab), and a shape measure free from size (SH = b/a). This method also allows working with another 20 indexes: 10 angles formed among the landmarks (A-J), the centre of the ellipse, and the larger radii (a); and 10 distances between the same points and the centre of the ellipse. Of the 24 characters supplied by the method, 19 were used in the analyses: a, b, size (SI) and shape (SH) of the wing, the angles θA - θJ, and the distances rF - rJ. The repeatability of landmarks (Lessells & Boag 1987) was always higher than 99%, indicating that the ellipse method is highly reliable.

The asymmetry values of the traits were calculated individually in each species and season as FA1 = mean |R–L| (Palmer 1994). The normality of the distribution was evaluated by skewness and kurtosis, and the significance of the difference between the real mean of deviation and value zero (representing symmetry) was tested by a T-test with the Bonferroni correction applied. FA was assumed in the case of a normal distribution of (R–L) with the mean zero, and directional asymmetry in the normal distribution of (R–L) with the mean different from zero. The difference in asymmetry level between seasons, for each species, individually, was also tested using a T-Test. All statistical analyses were performed using software SYSTAT 9.0.

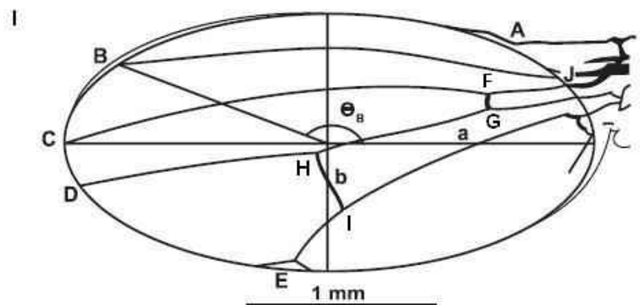

Figure 2. *Drosophila* wing and adjusted ellipse. The two director radii are a and b, respectively. The points A – J determine the junctions of veins to contour of the wing, or intersections of veins. For a given point, e.g. B, the angle (θB) is formed between the line that joins it to the centre of the ellipse and the major axis (Bitner-Mathé & Klaczko 1999).

Table 1. Indexes (FA_1 x 100) and type of asymmetry of four drosophilid species collected in the *Reserva Ecológica do IBGE* in different seasons. N: number of flies. The superscript "R" and "L" represent respectively right directional asymmetry and left directional asymmetry. Indices without superscript are not different from zero and represent fluctuating asymmetry.

Species	Season	N	a	b	θA	θB	θC	θD	θE	θF	rF	θG	rG	θH	rH	θI	rI	θJ	rJ	SI	SH
D. mercatorum	Dry	6	1.45^{R}	0.21	2.34^{R}	1.33	1.31	1.42	2.20	2.18^{R}	1.87	2.47	1.77	3.00	1.13	3.79^{L}	0.66	1.68^{R}	1.69	0.53	0.50^{L}
	Rainy	19	1.86	0.40	3.23	1.35	0.60	0.87	2.20	3.00	1.63	2.81	1.78	4.05	1.74	4.98	0.47^{R}	1.96	1.54	0.53	0.90
D. simulans	Dry	28	1.24	0.46	1.96	1.08^{R}	1.09	0.87	2.79^{R}	1.60	1.09	1.59	1.15	27.13	0.97	4.38	0.92	1.08	1.88	0.48	0.71
	Rainy	42	1.30	0.45	1.82	1.69	0.97^{R}	1.08	3.15	1.69	1.38	1.65	1.30	24.91	1.33	7.02	1.04	1.18	1.94	0.46	0.78
D. sturtevanti	Dry	30	1.37	0.38^{R}	1.64	2.04	0.91	0.99	2.40	1.46	1.12	1.13	1.18	21.22	0.61^{L}	4.12^{R}	0.52	1.26	1.62	0.56	0.67
	Rainy	32	1.29	0.53	2.17	1.39	0.74	0.78	3.03	1.61	1.01	1.66	1.23	24.03	0.79	5.75^{R}	0.88	1.33	1.57	0.64	0.66
Z. indianus	Dry	32	1.14^{L}	0.32	1.19	1.35	0.70	0.56	2.08	1.65	1.22	1.36	1.18	3.68	0.91	3.67	0.88	0.96	1.19	0.56^{L}	0.43
	Rainy	29	1.63	0.54	1.76	1.24	0.77	0.66	3.11	2.47	1.52	2.85	1.54	4.50	1.81	5.33	0.96	1.45	1.37	0.69	0.68

RESULTS

The indexes and asymmetry type for each species in the two seasons are shown in Table 1, where the R and L represent respectively right and left directional asymmetry, and indices without superscript represent fluctuating asymmetry. The amount of asymmetry varied considerably amongst characters, but the most asymmetrical traits were usually the angles θH and θI, and the least asymmetrical ones were b, SI, and SH (Figure 3). Regarding asymmetry type, a predominance of fluctuating asymmetry was observed, as well as some occurrences of directional asymmetry, mainly in the angle θI. As the large majority of traits showed fluctuating asymmetry in the four species, all of them were used in the subsequent analyses.

Although the visual inspection of the graphs suggests that the wing traits tend to be more asymmetrical in the rainy season (Figure 3), Bonferroni t-tests did not reveal any consistent difference in FA levels between the seasons.

DISCUSSION

Here we evaluated drosophilid populations under a stressful condition usually faced by them in nature: seasonal drought. It has been shown that all species investigated in this study suffer an accentuated population bottleneck in the dry season (Tidon 2006, Mata & Tidon 2013, Roque *et al*. 2013, Mata *et al*. 2015), indicating that, in some way, they are influenced by the low humidity that characterizes this season. However, our data did not show significant differences in the symmetry of flies collected in the dry and rainy seasons. This result can be due to different factors, such as experimental design, natural selection, or the real absence of the effect of season on flies symmetry.

Markow (1995) emphasizes that an incorrect experimental design can hide FA in conditions where it should happen. For that reason, in this study we adopted several methodological guidelines recommended in the literature (Palmer & Strobeck 1986, 1992, Palmer 1994, Lens *et al.* 2002). We tested the repeatability of the measures; the parameters of FA, the influence of the body size in the asymmetry, the indexes to assess FA, and the comparative tests were also selected carefully. Thus, we consider the results obtained in this study robust.

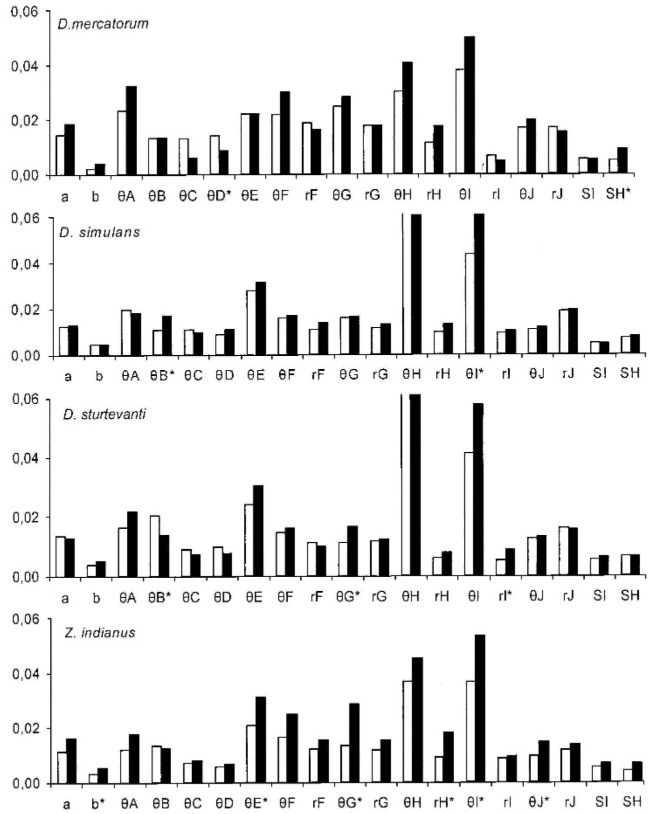

Figure 3. Fluctuating asymmetry (FA1) of 19 characters measured for four drosophilid species in the dry (white bars) and rainy (black bars) seasons. Asterisks show significant differences between seasons. (T-test, * $p < 0.05$).

It has been suggested that certain characters can reflect the relationship between stress and asymmetry more clearly than others (Lens *et al.* 2002, Coster *et al*. 2013). Accordingly, in *Drosophila ananassae,* the effects of thermal stress on FA seem to be trait and sex specific (Vishalakshi & Singh, 2009). However, studies on wing FA have shown contrasting results. Wing size FA was affected by the quality of host plants in the cactophilic species *D. buzzatii* and *D. koepferae* (Soto *et al.* 2008) and by thermal stress in *D. melanogaster* (Trotta *et al.* 2005). On the other hand, *D. melanogaster* exposed to pesticides showed no difference in FA wing length when compared to control flies (Antipin & Imasheva 2001, Hoffmann *et al.* 2005). Field studies focused on *D. serrata* in Australia (Jenkins & Hoffmann 2000) have also found no increased wing size FA in flies submitted to supposedly adverse conditions in nature. We suggest that wing FA responses could be stressor specific and differ between studies performed in the laboratory and field.

Wings are vital flight organs and interfere in male reproductive success since the females use the sound produced by the male's wing vibration to identify fitness and species (Menezes *et al*. 2013). Therefore, they are likely under selection in natural environments. In the Brazilian Savanna, selection should be stronger during the dry season, and it is possible that only the most symmetrical specimens reach the adult phase. This is called developmental selection and has already been successfully demonstrated by Polak *et al*. (2002). Alternatively, the similar wing asymmetry levels found in the dry and rainy seasons may mean that environmental drought does not affect the wing symmetry. Although our study added more data to the relationship between asymmetry and stress, this discussion seems to be far from being solved.

CONCLUSIONS

In short, this study did not confirm the hypothesis that drosophilids are more asymmetrical in the dry season, considered a more stressful season when compared to the rainy season. The possible reasons for these surprising results were discussed above, and we recommend that future research relating fluctuating asymmetry with developmental instability pay particular attention to planning and analysis of the data, as well as to the life history of the organisms studied. Following these guidelines, it should be possible to verify if there are certain groups of organisms or characters that are more suitable than others to evaluate asymmetry, or even if asymmetry is a good indicator only for certain types of stress. Hopefully, it will be possible to reach a consensus about the real usefulness of FA as an indicator of developmental instability.

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REFERENCES

Ambo-Rappe, R., Lajus, D.L. & Schreider, M.J. (2008) Increased heavy metal and nutrient contamination does not increase fluctuating asymmetry in the seagrass *Halophila ovalis*. *Ecological indicator* 8: 100-103.

Anciães, M. & Marini, M.A. (2000) The effects of fragmentation on fluctuating asymmetry in passerine birds of Brazilian tropical forests. *Journal of Applied Ecology* 37: 1013-1028. https://doi.org/10.1046/j.1365-2664.2000.00554.x

Antipin, M. I. & Imasheva, A. G. (2001) Genetic Variability and Fluctuating Asymmetry of Morphological Traits in Drosophila melanogaster Reared on a Pesticide-Containing Medium, Russian Journal of Genetics, 37: 247–252. https://doi: 10.1023/A:1009000925144

Beasley, D.A.E., Bonisoli-Alquati, A. & Mousseau, T. A. (2013) The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. *Ecological Indicators* 30: 218–226. https://doi.org/10.1016/j.ecolind.2013.02.024

Benítez, H. A. & Parra, L. E. (2011) Fluctuating asymmetry: A morpho-functional tool to measure development stability. *International Journal of Morphology* 29(4): 1459–1469. https://doi.org/10.4067/s0717-95022011000400066

Bjorksten, T.A., Pomiankowski, A. & Fowler, K. (2001) Temperature shock during development fails to increase the fluctuating asymmetry of a sexual trait in stalk-eyed flies. *Proceedings of the Royal Society of London Series B-Biological Sciences* 268(1475): 1503-1510. https://doi.org/10.1098/rspb.2001.1575

Bitner-Mathé, B.C. & Klaczko, L.B. (1999) Heritability, phenotypic and genetic correlations of size and shape of *Drosophila mediopunctata*. *Heredity* 83: 688-696. https://doi.org/10.1046/j.1365-2540.1999.00606.x

Clarke, G.M. (1995) Relationship between developmental stability and fitness: Application for conservation biology. *Conservation Biology* 9(1): 18-24.

Costa, R. N., Nomura, F. & Solé, M. (2017) Agropastoral activities increase fluctuating asymmetry in tadpoles of two neotropical anuran species. Austral Ecology 42: 801–809. https://doi.org/10.1111/aec.12502

Coster, G., van Dongen, S., Malaki, P., Muchane, M., Alcántara-Exposito, A., Matheve, H. & Lens, L. (2013) Fluctuating asymmetry and environmental stress: understanding the role of trait history. *PLoS ONE* 8(3): e57966. https://doi.org/10.1371/journal.pone.0057966

Dongen, S.V. (2006) Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. *Journal of Evolutionary Biology* 19(6): 1727-1743. https://doi.org/10.1111/j.1420-9101.2006.01175.x

Freire-Maia, N. & Pavan, C. (1949) Introdução ao estudo da Drosófila. Cultus 1, 1-71.

Galego, L.G.C. & Carareto, C.M.A. (2010) Scenario of the spread of the invasive species *Zaprionus indianus* Gupta, 1970 (Diptera, Drosophilidae) in Brazil. *Genetics and Molecular Biology* 33(4): 767–773. https://doi.org/10.1590/S1415-47572010005000080

Hoffmann, A.A., Woods, R.E., Collins, E., Wallin, K., White, A. & McKenzie, J.A. (2005) Wing shape versus asymmetry as an indicator of changing environmental conditions in insects. *Australian Journal of Entomology*, *44*(3): 233–243. https://doi.org/10.1111/j.1440-6055.2005.00469.x

Hosken, D.J., Blanckenhorn, W.U. & Ward, P.I. (2000) Developmental stability in yellow dung flies (*Scathophaga stercoraria*): fluctuating asymmetry, heterozygosity and environmental stress. *Journal of Evolutionary Biology* 13(6): 919-926. https://doi.org/10.1046/j.1420-9101.2000.00239.x

Imasheva, A.G. & Bubliy, O.A. (2003) Quantitative variation of four morphological traits in *Drosophila melanogaster* under larval crowding. *Hereditas* 138(3): 193-199. https://doi.org/10.1034/j.1601-5223.2003.01727.x

Jenkins, N.L. & Hoffmann, A. A. (2000) Variation in morphological traits and trait asymmetry in field *Drosophila serrata* from marginal populations. *Journal of Evolutionary Biology* 13(1): 113–130. https://doi.org/10.1046/j.1420-9101.2000.00149.x

Klaczko, L.B. & Bitner-Mathé, B.C. (1990) On the edge of a wing. *Nature* 346(6282): 321. https://doi.org/10.1038/346321a0

Klingenberg, C.P. (2015) Analyzing fluctuating asymmetry with geometric morphometrics: Concepts, methods, and applications. *Symmetry* 7(2): 843–934. https://doi.org/10.3390/sym7020843

Klingenberg, C.P. (2019) Phenotypic plasticity, developmental instability, and robustness: The concepts and how they are connected. *Frontiers in Ecology and Evolution* 7: 1–15. https://doi.org/10.3389/fevo.2019.00056

Koeberle, A.L., Arismendi, I., Crittenden, W., Leer, D. & Noakes, D.L.G. (2020) Fluctuating asymmetry of adult Chinook Salmon (*Oncorhynchus tshawytscha*) otoliths from wild and hatchery origins. *Aquatic Ecology* 54(1): 431–446. https://doi.org/10.1007/s10452-019-09733-0

Lens, L.S., Van Dongen, S., Kark, S. & Matthysen, E. (2002) Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? *Biological Reviews* 77(1): 27-38. https://doi.org/10.1017/S1464793101005796

Lessells, C.M. & Boag, P.T. (1987) Unrepeatable repeatabilities: A common mistake. *The Auk* 104(1): 116-121. https://doi.org/10.2307/4087240

Manfrin, M.H., Prado, P.R.R. & Sene, F.M. (1997) Analysis of sound components of sexual courtship of two subspecies of *Drosophila mercatorum* (Diptera, Drosophilidae). *Revista Brasileira de Biologia* 57: 349-355.

Menezes, B.F., Vigoder, F.M., Peixoto, A.A., Varaldi, J., & Bitner-Mathé, B.C. (2013) The influence of male wing shape on mating success in *Drosophila melanogaster*. *Animal Behaviour*, 85(6): 1217-1223

Markow, T.A. (1995) Evolutionary Ecology and Developmental Instability. *Annual Review of Entomology* 40: 105-120. https://doi.org/10.1146/annurev.en.40.010195.000541

Mata, R.A., McGeoch, M. & Tidon, R. (2010a) Drosophilids (Insecta, Diptera) as tools for conservation biology. *Natureza & Conservação* 8(1): 60–65. https://doi.org/10.4322/natcon.00801009

Mata, R.A., Tidon, R., Côrtes, L.G., de Marco, P. & Diniz-Filho, J.A.F. (2010b) Invasive and flexible: Niche shift in the drosophilid *Zaprionus indianus* (Insecta, Diptera). *Biological Invasions* 12(5): 1231–1241. https://doi.org/10.1007/s10530-009-9542-0

Mata, R.A. & Tidon, R. (2013) The relative roles of habitat heterogeneity and disturbance in drosophilid assemblages (Diptera, Drosophilidae) in the Cerrado. *Insect Conservation and Diversity* 6(6): 663–670. https://doi.org/10.1111/icad.12020

Mata, R.A., Valadão, H. & Tidon, R. (2015) Spatial and temporal dynamic of drosophilids larval assemblages associated to fruits. *Revista Brasileira de Entomologia* 59(1): 50–57. https://doi.org/10.1016/j.rbe.2015.02.006

Moller, A.P. & Swaddle, J.P. (1997) *Asymmetry,* developmental *stability and evolution*. Great Britain: Oxford University Press, 304 pp.

Mohr, S.E. (2018) *First in fly: Drosophila research and Biological Discovery*. Harvard University Press, 272 pp.

Oliveira, P.S. & Marquis, R.J. (2002) *The Cerrados of Brazil. Ecology and natural history of a Neotropical savanna*. New York: Columbia University Press, 450 pp.

Palmer, A.R. (1994) Fluctuating asymmetry analyses: A primer. In: Markow, T.A. (Ed.) *Developmental instability: its origins and evolutionary implications*. Kluwer, Dordrecht, Netherlands. pp. 335-364.

Palmer, A.R. & Strobeck, C. (1986) Fluctuating asymmetry - measurement, analysis, patterns. *Annual Review of Ecology and Systematics* 17: 391-421. https://doi.org/10.1146/annurev.es.17.110186.002135

Palmer, A.R. & Strobeck, C. (1992) Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. *Acta Zoologica Fennica* 191: 55-70.

Pertoldi, C. & Kristensen, T.N. (2015) A new fluctuating asymmetry index, or the solution for the scaling effect? *Symmetry* 7(2): 327–335. https://doi.org/10.3390/sym7020327

Pinheiro, F., Diniz, I.R., Coelho, D. & Bandeira, M.P.S. (2002) Seasonal pattern of insect abundance in the Brazilian Cerrado. *Austral Ecology* 27(2): 132-136. https://doi.org/10.1046/j.1442-9993.2002.01165.x

Polak M., Opoka, R. & Cartwright, I.L. (2002) Response of fluctuating asymmetry to arsenic toxicity: support for the developmental selection hypothesis. *Environmental Pollution* 118(1): 19-28. https://doi.org/10.1016/S0269-7491(01)00281-0

Powell, J.R. (1997) *Progress and Prospects in Evolutionary Biology: The Drosophila Model*. Oxford University Press, New York, 580 pp.

Ribeiro, J.F. & Walter, B.M.T. (1998) Fitofisionomias do Bioma Cerrado. In: Sano, S.M. & Almeida, S.P. (Eds.) *Cerrado: ambiente e flora*. Planaltina: EMBRAPA-CPAC, pp. 89-166.

Roque, F., Mata, R.A. & Tidon, R. (2013) Temporal and vertical drosophilid (Insecta; Diptera) assemblage *fluctuations* in a neotropical gallery forest. *Biodiversity and Conservation* 22(3): 657–672. https://doi.org/10.1007/s10531-013-0433-4

Roque, F., Mencarini, L., Leão, B.F.D., Delgado, M.N. & Tidon, R. (2016) Three new records of drosophilids for the Brazilian Savanna. *Drosophila Information Service* 99: 2015–2016.

Sene, F.M., Val, F.C., Vilela, C.R. & Pereira, M.A.Q.R. (1980) Preliminary data on the geographical distribution of *Drosophila* species within morphoclimatic domains of Brazil. *Papéis Avulsos de Zoologia* 33: 315-326.

Soto, I.M., Carreira, V.P., Soto, E.M. & Hasson, E. (2008) Wing morphology and fluctuating asymmetry depend on the host plant in cactophilic *Drosophila*. *Journal of Evolutionary Biology* 21(2): 598–609. https://doi.org/10.1111/j.1420-9101.2007.01474.x

Tidon, R. (2006) Relationships between drosophilids (Diptera, Drosophilidae) and the environment on two contrasting tropical vegetations. *Biological Journal of the Linnean Society* 87(2): 233-247. https://doi.org/10.1111/j.1095-8312.2006.00570.x

Tidon, R., Leite, D.F. & Leão, B.F.D. (2003) Impact of the colonisation of *Zaprionus* (Diptera, Drosophilidae) in different ecosystems of the Neotropical region: 2 years after the invasion. *Biological Conservation* 112(3): 299-305. https://doi.org/10.1016/S0006-3207(02)00322-1

Tidon-Sklorz, R. & Sene, F.M. (1999) Diptera: *Drosophila*. In: Brandão, C.R.F. & Cancello, E.M. (Eds.) *Biodiversidade do Estado de São Paulo: I síntese do conhecimento ao final do século XX*. São Paulo, vol. 5, pp. 245-261.

Trotta, V., Calboli, F.C.F., Garoia, F., Grifoni, D., & Cavicchi, S. (2005) Fluctuating asymmetry as a measure of ecological stress in *Drosophila melanogaster* (Diptera: Drosophilidae). *European Journal of Entomology 102*(2): 195–200. https://doi.org/10.14411/eje.2005.031

Vangestel, C. & Lens, L. (2011) Does fluctuating asymmetry constitute a sensitive biomarker of nutritional stress in house sparrows (*Passer domesticus*)? *Ecological Indicators* 11(2): 389–394. https://doi.org/10.1016/j.ecolind.2010.06.009

Vilela, C.R. (1983) A revision of the *Drosophila repleta* species group (Diptera, Drosophilidae). *Revista Brasileira de Entomologia* 27: 1-114.

Vilela, C.R. (1999) Is *Zaprionus indianus* Grupta, 1970 (diptera, drosophilidae) currently colonizing the Neotropical region? *Drosophila Information Service* 82: 37-39.

Vishalakshi, C., & Singh, B.N. (2009) Fluctuating asymmetry in hybrids of sibling species, *Drosophila ananassae* and *Drosophila pallidos*a, is trait and sex specific. *Journal of Heredity* 100(2): 181–191. https://doi.org/10.1093/jhered/esn094

Wolda, H. (1988) Insect seasonality: why? *Annual Review of Ecology and Systematics* 19: 1–18. https://doi.org/10.1146/annurev.ecolsys.19.1.1