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Isolated frogs in a crowded world: Effects of human-caused habitat loss on frog heterozygosity and fluctuating asymmetry

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ABSTRACT

It is important to develop research methods that will detect deterioration in population health before severe declines occur. Amphibian population declines are widespread; the main causes are anthropogenic and include habitat fragmentation due to agriculture, mining, fires, and urban development. Brazil is the richest country in species of amphibians, and the Brazilian regions with the greatest amphibian diversity are experiencing relatively high rates of habitat destruction. We tested the use of measurements of fluctuating asymmetry (FA) taken on adult frogs, as well as heterozygosity estimates in frog populations, to detect anthropogenic stress. We hypothesized that greater human occupancy in the landscape might result in more stressful conditions for amphibians, which would be reflected in high FA levels and/or reduced heterozygosity. We conducted this study at the Espinhaço mountain range in southeastern Brazil, using an endemic species (*Bokermannohyla saxicola*, Hylidae) as a model. We found that adult frog FA levels differed among localities but did not find significant correlations between human modification of the landscape and FA levels. In the subsample of localities for which we had genetic data, heterozygosity was significantly inversely correlated with FA in adult frogs, and heterozygosity decreased with increasing human occupancy in the landscape, except for populations with marginal distribution within the species range that already had very low heterozygosity. Our major finding was that reduced heterozygosity may be caused by population isolation resulting from human occupancy (among other causes) and is correlated with increased fluctuating asymmetry, so that either may indicate higher levels of stress in populations of *B. saxicola*.

1. Introduction

Amphibian population declines have been causing concern since the 80s, when they were noticed to be occurring worldwide (Blaustein and Wake, 1990). There are several causes that together can lead to amphibian declines, and also cases that remain to be explained, since some declines occur in apparently pristine habitats (Gardner, 2001; Collins and Storfer, 2003). Relatively little is known about the status of amphibian populations in Latin American countries due to insufficient data on species distribution and population dynamics, combined with high levels of species diversity (Myers et al., 2000; Young et al., 2001). In Brazil, most recorded cases have occurred in the Atlantic Forest, where amphibians have been comparatively intensely studied (e.g. Heyer et al., 1988; Weygoldt, 1989; Bertoluci and Heyer, 1995; Guix et al., 1998; Pombal and Haddad, 1999; Izecksohn and Carvalho-e-Silva, 2001).

Brazil is presently known to contain 1026 species of amphibians, most of them described in the last 40 years (Segalla et al., 2014). The main threat to amphibian conservation in Brazil is habitat destruction and fragmentation, as a consequence of deforestation, agriculture, cattle ranching, fires, and urbanization (Silvano and Segalla, 2005; Becker et al., 2007). Many habitats in southeastern Brazil are suffering the impacts of tourism and human occupancy, which may cause great habitat loss and fragmentation in the near future (Myers et al., 2000), with potential consequences for amphibian populations. Field monitoring techniques may only detect population declines when they are already underway. Predictive techniques such as monitoring developmental instability or genetic variability of populations could detect problems faced by a population before it shows evidence of declines, allowing preventive actions to be taken in time (Avise, 1994; Alford et al., 1999, 2007; Kellner and Alford, 2003).

Developmental stability is the ability of a genotype to repeatedly produce the same phenotype under the same environmental conditions, and often reflects the general health of populations (Alford et al., 1999). However, phenotypes expressed by organisms may depart from their genetically-programmed targets; such departures are termed developmental instability (DI). For most traits, we do not know exactly what the target phenotype is, and therefore we cannot determine how the expressed phenotype differs from it. However, most bilaterally symmetrical traits appear to be programmed for perfect symmetry, allowing

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DI to be measured as the degree of asymmetry of such traits (Palmer and Strobeck, 2003). DI can be higher in individuals that experience higher levels of stress during growth and development and can indicate stressed populations that may be at greater risk of decline (Alford et al., 1999, 2007).

Fluctuating asymmetry (FA, in which differences between right and left sides are normally distributed about a mean of zero) is often considered as the most appropriate type of asymmetry to use as a measure of developmental instability. Fluctuating asymmetry differs from directional asymmetry (DA), in which differences between right and left sides are normally distributed about a mean that is significantly different from zero, and antisymmetry (AS), in which differences between right and left sides are distributed about a mean of zero, but the distribution is bimodal or leptokurtic instead of normal (Palmer and Strobeck, 2003). DA and AS are believed to arise from a complex mixture of genetic and non-genetic causes (Palmer and Strobeck, 1992, 2003).

Genetic stress can cause fluctuating asymmetry to increase (Parsons, 1992). Heterozygosity is a measure of genetic diversity and is frequently associated with population fitness (Avise, 1994). The lowest levels of DI are thought to occur when heterozygosity levels allow populations to be healthy and not susceptible to developmental stress (Kellner and Alford, 2003). Associations between low genetic diversity and high levels of FA have been shown for some groups, such as fish (Iguchi et al., 2005) and cats (Wayne et al., 1986), but not in all cases (Vollestad and Hindar, 2001), so there is no universal heterozygosity/FA association (Leamy and Klingenberg, 2005).

Habitat loss is one of the many factors that can influence genetic diversity and FA. Studies of the relationship between FA and habitat disturbance in amphibian populations are inconclusive, some pointing to higher levels of FA in disturbed than in undisturbed sites (Wright and Zamudio, 2002; Söderman et al., 2007), and others finding little evidence supporting an increase of FA with environmental disturbance (Delgado-Acevedo and Restrepo, 2008).

Bokermannohyla saxicola (Bokermann, 1964) is a treefrog that lives in the highlands of the Espinhaço Mountain range, southeastern Brazil (Frost, 2015). It breeds in streams, and tadpoles take at least five months to develop (Eterovick and Sazima, 2004). Populations of this treefrog can show genetic differentiation when at least 2.5 km apart (Eterovick et al., 2009). We previously found that FA levels in tadpoles can vary among *B. saxicola* populations, but found no association of FA with anthropogenic modification of the landscape (Eterovick et al., 2015). We hypothesized that tadpoles may be likely to reflect more localized impacts such as those on water quality, with increased FA resulting, for instance, from water contaminants (e.g., Costa and Nomura, 2015). It is also possible that the genetic backgrounds of different populations affect how FA levels respond to local levels of stress (Eterovick et al., 2015). Post metamorphic frogs occupy riparian habitats and are thus likely to use a larger area than tadpoles (see Semlitsch and Bodie, 2003); we therefore hypothesized that the FA levels of adult frogs might be a better measure of human impacts at the landscape level than were the FA levels of tadpoles. We also examined whether levels of genetic diversity were correlated with landscape levels of human impacts and with frog FA levels. We hypothesized that human occupancy in the landscape would hamper frog movements by disrupting migration routes, and that this would be reflected in reduced heterozygosity in local populations. We also hypothesized that populations with reduced heterozygosity would show greater levels of FA.

2. Materials and methods

2.1. Study site

The Espinhaço Mountain range extends for about 1000 km from Bahia to Minas Gerais states in eastern Brazil (Derby, 1906). It acts as a barrier between the Atlantic Forest and the open Cerrado formations in Central Brazil and it also divides major water basins. These features result in varied vegetation formations, and in high levels of diversity and endemism in many taxonomic groups (Giulietti and Pirani, 1988). A total of 105 anuran species have been recorded for the Espinhaço, 33 of which are endemic (Leite et al., 2008). Highlands (above 1000 m) where *B. saxicola* occurs are relatively well preserved compared to lower areas, probably because the hilly terrains are not attractive to human activities such as agriculture and cattle raising (Eterovick et al., 2015; PCE pers. obs.).

2.2. Image analyses using GIS

We used RapidEye™ satellite images from 2010 made available by the Instituto Estadual de Florestas de Minas Gerais (IEF-MG) to quantify human occupancy in the landscape. The images have a spatial resolution of 6.5 m (Tyca et al., 2005) and, after orthorectification, the resolution becomes 5 m per pixel of image, corresponding to a scale of 1:25,000 (Cheng and Sustera, 2009).

Image analyses were performed as in Eterovick et al. (2015). We used the same buffers around sampling points (2.5 km and 368 m radii); these are based on the area likely to be covered by local populations of *B. saxicola* (Eterovick et al., 2009) and the estimated maximum core terrestrial habitat for frogs, as suggested in the review by Semlitsch and Bodie (2003), respectively. We established classes of soil cover within the area of each buffer and calculated percentage of those with human influence using the Interactive Supervised Classification function in the software ARCGIS (see details in Eterovick et al., 2015). We considered arboreal vegetation and rock/montane meadows as natural habitats, and altered vegetation (pastures, areas occupied by introduced herbaceous plants), agriculture, and constructed areas (human settlements, roads, exposed soil around human settlements, mines) as anthropogenic habitats. We checked habitat classification in the field. One of us (PCE) and a colleague (F.S.F. Leite, pers. comm.) have been conducting research throughout the Espinhaço Range since 1998, with more intensive and widespread sampling during 2006–2011. Although there was an interval up to 8 years between frog collection and map analyses, we are familiar with the localities included in this study and we know that no major changes in area used by humans that could influence our results occurred during this period in those areas encompassed by our buffers. The 2.5 km buffers of sampling points overlapped for points 2 and 3; 6, 8 and 9; 10–13; 14–16; 18 and 19; so we merged them and pooled the samples of those sampling points, resulting in 15 buffers.

2.3. Heterozygosity

We used 14 highly polymorphic microsatellite sites described for *B. saxicola* (Eterovick et al., 2012) to estimate heterozygosity at each sampled locality. The same dataset is currently being used to study spatial population structure of *B. saxicola* populations (Eterovick et al., unpublished data). We obtained tissue samples from 191 individuals from 13 localities, 10 of those corresponding to localities from which we also collected asymmetry data (Table 1). Tissue samples were obtained from 2007 to 2009. We isolated DNA using the Wizard Genomic DNA Purification Kit and protocol (Promega). We quantified DNA samples with a Nanodrop ND-1000 spectrophotometer and diluted samples to 20 ng/μl for PCR reactions. We ran PCR reactions with buffer to 1× final concentration, dNTPs (0.60 mM), MgCl (1.5 mM), primers (0.20 μM), Taq polymerase (0.5 units), 3 μl of DNA template, and water to a final volume of 10 μl. Reactions started with 96 °C for 2 min, 35 cycles of 96 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, then 72 °C for 10 min. We checked PCR products on 3% agarose gels visualized under UV light after staining with ethidium bromide. We genotyped microsatellites labeling their primers with fluorescent dyes 6FAM, HEX and NED for multiplexing on the 3730xl DNA Analyzer. We analyzed the 14 microsatellite loci for 191 individuals with the