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Variation of thermal parameters in two different color morphs of a diurnal poison toad, *Melanophryniscus rubriventris* (Anura: *Bufonidae*)

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ABSTRACT

We study the variation in thermal parameters in two contrasting populations Yungas Redbelly Toads (*Melanophryniscus rubriventris*) with different discrete color phenotypes comparing field body temperatures, critical thermal maximum and heating rates. We found significant differences in field body temperatures of the different morphs. Temperatures were higher in toads with a high extent of dorsal melanization. No variation was registered in operative temperatures between the study locations at the moment of capture and processing. Critical thermal maximum of toads was positively related with the extent of dorsal melanization. Furthermore, we founded significant differences in heating rates between morphs, where individuals with a high extent of dorsal melanization showed greater heating rates than toads with lower dorsal melanization. The color pattern-thermal parameter relationship observed may influence the activity patterns and body size of individuals. Body temperature is a modulator of physiological and behavioral functions in amphibians, influencing daily and seasonal activity, locomotor performance, digestion rate and growth rate. It is possible that some growth constraints may arise due to the relationship of color pattern-metabolism allowing different morphs to attain similar sizes at different locations instead of body-size clines.

1. Introduction

Many studies addressing the adaptive significance or ecological implications of the coloration in amphibians have focused on how the variation translates into predator avoidance (Hoffman and Blouin, 2000; Rudh and Qvarnström, 2013; Toledo and Haddad, 2009). Aposematic anurans that are chemically defended by sequestered alkaloids advertise their defenses to predators via warning signals that are most often bright colors combined with black patterns (Amézquita et al., 2013; Bonansea and Vaira, 2012; Mochida, 2009). Empirical studies show that predators avoid attacking such patterned models, implying that appearance promotes the recognition of undesirable prey (Noonan and Comeault, 2009). However, positive results for the significance of particular color patterns as warning signals do not rule out other functions of coloration (Stevens and Ruxton, 2012). If selection influences the evolution of color traits, there may be conflicting pressures that modulate color expression resulting in a compromise in the final coloration pattern (Mochida, 2011). Conspicuousness of the coloration pattern is known to exhibit considerable phenotypic variation in amphibians, suggesting that color expression may be influenced by many selection pressures (Rudh and Qvarnström, 2013). In some cases, the coloration pattern may be a compromise between several functions (i.e. warning signals, thermoregulation, crypsis, immune system function, mate recognition) although the complexity of these interactions makes it difficult to fully understand the functional significance of coloration (Stevens and Ruxton, 2012).

The Yungas Redbelly Toad, *Melanophryniscus rubriventris*, is an endemic anuran of the Yungas Andean forest, distributed in northwestern Argentina and southern Bolivia. This species exhibits a geographic color polymorphism with the presence of marked variation in the extent of bright coloration. Populations vary from uniform olive color to black dorsal patterns, while others exhibit a vivid red dorsum (Bonansea and Vaira, 2012). Despite huge differences

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in bright coloration, all populations had similar amounts of skin alkaloids and consequently were suspected to be equally distasteful, constituting a case of aposematic polymorphism (Garraffo et al., 2012). What maintains this discrete polymorphism in these aposematic populations is an untested question in this species.

Identifying functional differences among color morphs seems central to understand the ecological or evolutionary significance of the maintenance of such discrete color polymorphisms in this species. Physiological functions of coloration besides the visual warning function should be further explored and might reveal other mechanisms that are critical for understanding color pattern variation (Hegna et al., 2013). Some frogs are known to vary in their efficiency of thermoregulation depending on their coloration, where darker individuals experimentally exposed to the sun reach higher body temperatures than lighter individuals (Vences et al., 2002). Therefore, two individuals with differences in dorsal melanization, but with similar conditions (e.g. posture, microhabitat) can reach thermal equilibrium at different times (Porter and Gates, 1969).

The aim of this study was to relate the observed color variation of the brightest and darkest color morphs of *M. rubriventris* with their thermoregulatory efficiency. Thus, we studied the variation in thermal parameters in two contrasting populations of Yungas Redbelly Toad with different discrete color phenotypes by comparing field body temperatures, critical thermal maximum and heating rates.

2. Methods

2.1. Study area and species

We conducted our study during February 2013, in two localities from Jujuy province, northwestern Argentina (Fig. 1): Angosto de Jaire (S 24.0097; W 65.3817; altitude: 1665 m a.s.l) and Abra Colorada (S 23.6812; W 64.9122; altitude: 1722 m a.s.l), in coincidence with the reproductive period of the species. Both study areas support well-structured montane forests with high vegetation species richness and vegetation density, but are slightly disturbed because of former clearings and the occasional presence of livestock. The entire distribution of this species is upland areas (1000 to 2000 m a.s.l) of a typical subtropical humid montane forest (Ecoregion of Southern Andean Yungas *sensu* Olson et al., 2001).

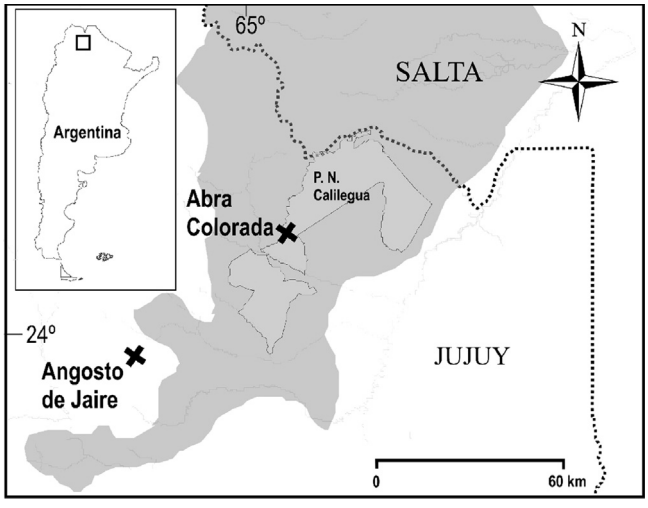

Fig. 1. Location of the studied populations of *Melanophryniscus rubriventris* in Jujuy province, NW Argentina. The larger gray shaded area is the Las Yungas Biosphere Reserve; the small gray area is the National protected area (P.N.).

M. rubriventris is a small diurnal aposematic toad, that is relatively common in primary and secondary montane forests of NW Argentina and breeds after heavy rains from November to February (Vaira, 2005). Breeding activities involve large aggregations with short and explosive reproductive events that vary highly in numbers among years where toads use shallow temporary ponds to lay eggs (Vaira, 2005). This species shows marked geographic variation in dorsal coloration with a variety of discrete morphs, from bright orange coloration covering the head, shoulders, and flanks over a black background; to a complete olive to black dorsal coloration (see Bonansea and Vaira (2012) for more detailed morph descriptions). We selected individuals from two populations differing mainly in the extent of black patches (hereafter referred as dorsal melanism).

A total of 34 toads were captured by hand and measured during a single day at each site (12 from Angosto de Jaire, and 22 from Abra Colorada).

2.2. Determination of the extent of dorsal melanization

Before taking body temperature measurements (see below), every specimen was photographed in the field from the dorsal view with a digital camera (Nikon® D80, Japan) with normal lens (Sigma® 50 mm, Japan). Those images were then imported into the public domain image processing program ImageJ 1.47 (Rasband, 2012) to calculate the extent of black dorsal skin. Images were converted to grayscale of 8 bits, and outlines of the entire body area and black areas were drawn using manual threshold settings. The extent of the dorsal melanization was then calculated from outline areas and determined as percentages of the total area of the dorsal view of toad. Individuals used in the study were well within the range of variation observed in source populations (Bonansea and Vaira, 2012). Individuals from Angosto de Jaire were characterized as high dorsal melanization (HM), while the individuals from Abra Colorada were characterized as low dorsal melanization (LM) (Fig. 2).

2.3. Determination of field body temperature and environmental parameters

Body temperature (T_b) of free-ranging toads was measured at the time of capture (catheter probes TES TP-K01) with a digital thermometer TES 1312 (TES Electrical Electronic Corp., Taipei, Taiwan, ± 0.1 °C). Toads were gripped by a forelimb to minimize heat exchange during manipulations and a thermocouple was

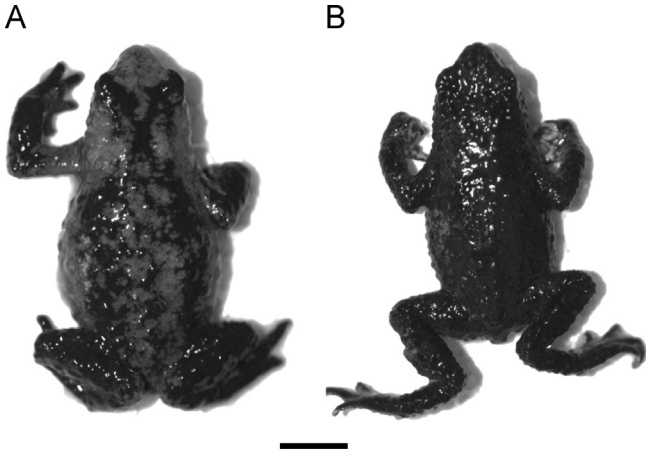

Fig. 2. Two males of *Melanophryniscus rubriventris* showing different amount of dorsal melanization. (A) Typical toad with a low dorsal melanization (LM) from Abra Colorada, (B) Typical toad with a high dorsal melanization (HM) from Angosto de Jaire, Jujuy, Argentina. Scale bar = 1 cm.