

## Behavioral Avoidance of Ultraviolet-B Radiation by Two Species of Neotropical Poison-Dart Frogs

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### ABSTRACT

Many animals, plants, and microorganisms are harmed by ultraviolet-B radiation. In particular, several members of class amphibia are negatively affected by exposure to ultraviolet-B radiation. Exposure to ultraviolet-B radiation can cause death or various types of sublethal damage in amphibians. One mechanism to lessen the effect of harmful ultraviolet-B radiation is to limit exposure to sunlight behaviorally. Few studies have examined the behavioral sensitivity of adult amphibians to ultraviolet-B radiation. Using both field experiments and field observations, we found that two species of diurnal poison-dart frogs in Costa Rica (*Dendrobates pumilio*, *D. auratus*) consistently preferred areas in the field and within experimental testing chambers that offered low levels of ultraviolet-B radiation. In field observations, vocalizing *D. pumilio* were found at locations with significantly lower levels of ambient ultraviolet-B compared to random locations throughout their natural habitat. Ultraviolet-B avoidance behavior may be an important behavioral response for tropical frogs in light of recent evidence suggesting a significant increase in the levels of ambient ultraviolet-B radiation in the tropics over the past decade.

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*Key words:* amphibians; avoidance behavior; Costa Rica; lowland rain forest; poison-dart frog; UV-B.

INCREASING ULTRAVIOLET-B (UV-B; 280–315 nm) RADIATION, due to stratospheric ozone depletion, is thought to be harming many plants, animals, and microorganisms (Häder 1993, Tevini 1993, Cockell 2001) and is believed to be one of several factors contributing to the global decline of amphibians (Stuart *et al.* 2004, Lannoo 2005). Exposure of amphibians to UV-B decreases hatching success and survivorship of embryos, induces sublethal effects, increases susceptibility to lethal pathogen outbreaks, and causes changes in behavior including locomotion, orientation, and antipredator responses in larvae and post-metamorphic stages (Blaustein *et al.* 2000, Kats *et al.* 2000, Kiesecker *et al.* 2001, Blaustein & Kiesecker 2002). Most research on the effect of UV-B radiation on amphibians has focused on mortality and development of embryos and larvae in temperate regions (Blaustein *et al.* 1998, Blaustein & Kiesecker 2002). It is, however, the Neotropics that are currently experiencing the fastest rate of amphibian decline (Middleton *et al.* 2002, Stuart *et al.* 2004). Despite this, few studies examining UV-B on tropical amphibians have been conducted (Stuart *et al.* 2004). Many species of tropical frogs frequent light gaps, breed in exposed areas within the rain forest (especially disturbed areas), or inhabit streams that are exposed to sunlight (Middleton *et al.* 2002, Savage 2002). Yet, UV-B radiation has rarely been considered as a prominent factor affecting tropical frogs. Many tropical frogs seek light gaps for mating purposes. Male poison-dart frogs (*Dendrobatidae*) perch in light

gaps or other open areas in the forest understory, presumably to maximize call effectiveness (Pröhl & Hödl 1999, Savage 2002). It is possible that dendrobatid frogs are experiencing conflicting selection pressures in their chosen microhabitats as maximizing call effectiveness conflicts with the need to reduce exposure to UV-B. With increasing UV-B levels in these regions, seeking out light gaps from which to issue mate calls may expose males to doses of UV-B radiation that have only recently become harmful (within the last 100 yr). Behavioral sensitivity to UV-B radiation may allow dendrobatid frogs to avoid damaging doses of UV-B even while frequenting favored microhabitats where UV-B is high. Selection should favor frogs that maximize vocalization in light gaps and other suitable microhabitats while minimizing their exposure to potentially damaging UV-B radiation. We examined UV-B avoidance behavior in two species of poison-dart frog, *Dendrobates pumilio* (the strawberry poison-dart frog) and *D. auratus* (the green poison-dart frog) using enclosures in the field, and by surveying UV-B radiation levels at natural calling sites of adult male *D. pumilio* in Costa Rica.

### METHODS AND RESULTS

UV-B CHOICE TESTS.—Experiments were conducted in a lowland tropical rain forest clearing at La Selva Biological Research Station in Costa Rica (10°43' N, 83°98' W; 35 m asl). Choice tests were conducted between 1100 and 1300 h over a

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six day period, when ambient UV-B levels were between 5 and  $15 \mu\text{W}/\text{cm}^2$ . Choice tests were conducted for 39 adult individuals of *D. pumilio* (mean =  $19.80 \text{ mm} \pm 0.14$ ) and 11 adult individuals of *D. auratus* (mean =  $33.5 \text{ mm} \pm 1.0$ ). Ambient UV-B levels (280–315 nm) were measured with a PMA2102 Outdoor UV-B meter (Solar Light Co., Philadelphia, PA, U.S.A.).

All animals were collected between 0800 and 1000 h on the same day as the experimental trials, along paved trails throughout primary growth forest. Animals were collected and maintained under government permit no. 19163 from the Ministerio del Ambiente y Energía de Costa Rica. Animals were held individually in separate containers (commercial opaque plastic chambers with perforated sides for air flow,  $21 \times 34 \text{ cm}$ ) with adequate moisture, space, and air, in a laboratory room ( $\sim 24^\circ\text{C}$ ) for no longer than 5 h after capture. Containers were kept in shady, cool conditions before and after outdoor experimental trials. Care was taken to return subjects to the approximate location of their capture, as these animals exhibit pronounced territoriality (Pröhl & Berke 2001). Sampling was conducted along dissimilar, separated stretches of paved trails to avoid possible recapture.

Experimental chambers were fitted with lids one side of which was constructed with Mylar filter (transmits about 3% ambient UV-B) and the other side of acetate filter (transmits about 82% ambient UV-B). The chamber floors were lined with moist paper towels to offer moisture and traction for animals during trials. Substrate did not offer the animals refuge or shade. We measured solar irradiance (400–1100 nm) transmitted through each filter using a Li-Cor 185B quantum/radiometer/photometer with a pyranometer sensor, and found no significant difference (Mylar:  $462 \pm 72 \text{ Watts}/\text{m}^2$ , acetate:  $474 \pm 75 \text{ Watts}/\text{m}^2$ ; paired Student's *t*-test,  $P = 0.16$ ).

Each chamber held one adult frog per 10-min trial. Animals were placed at the center of the chamber floor, under the lid at the partition between Mylar and acetate filters. Location of each animal was observed and recorded each minute for 10 min. Orientation of chambers was randomized at the start of trials, and all chambers were rotated 180 degrees after each 10-min trial to control for possible effects of chamber orientation.

The percentage data for the number of observations under Mylar was arcsine-transformed to correct for non-normal distribution, and transformed data were compared to a random distribution using a one-tailed Student's *t*-test.

Both species of poison-dart frogs spent significantly more time under the Mylar (UV-B inhibiting shield) than would be expected by chance (*D. pumilio*:  $57.40 \pm 4.17\%$ ,  $N = 39$ ,  $P = 0.04$ ; *D. auratus*:  $72.70 \pm 4.68\%$ ,  $N = 11$ ,  $P < 0.01$ ). Air and substrate temperatures within the experimental chambers did not differ under the filters on either side of the chamber (Mean air temperatures  $t_{10} = 1.19$ ,  $P = 0.26$ ; Mean substrate temperatures,  $t_{10} = 0.40$ ,  $P = 0.70$ ). Therefore, significant differences in body temperatures are unlikely for test animals held under the filters.

OBSERVATIONS AT CALLING VERSUS RANDOM SITES.—We compared the ambient UV-B levels at random locations to locations where adult *D. pumilio* males were observed calling throughout primary

and secondary lowland rain forest at La Selva Biological Station. Coordinates for random locations along the main paved trail were generated *a priori* using a random number table. An additional random number was chosen between 1 and 20 to dictate the distance (m) perpendicular to the main trail where UV-B measurements were recorded. Random sites were chosen along the main trail for survey tractability, and since frogs are rarely found resting in the middle of established trails through rain forest. At each of these random locations we recorded ambient UV-B levels where adult males may likely perch to project their call (such as fallen logs or at the buttresses of trees; Savage 2002). Measurements were taken at these random sites regardless of habitat heterogeneity (*e.g.*, tree stumps, buttresses, sites of natural disturbance). Thus, the UV-B probe was placed up to 1 m off the ground (buttresses) for some readings, consistent with observed perching sites of calling *D. pumilio*.

Along these trails we also recorded ambient UV-B levels for any calling adult *D. pumilio* males encountered. Measurements were taken at the exact location where animals were observed vocalizing. Data were collected between 1100 h and 1300 h, over the same six day period when choice tests were conducted. Data were analyzed using an unpaired Student's *t*-test.

While randomly sampled microhabitats in the rain forest received low levels of UV-B ( $0.72 \pm 0.13 \mu\text{W}/\text{cm}^2$ ,  $N = 50$ ) between 1100 h and 1300 h, microhabitats where *D. pumilio* were calling showed UV-B levels more than six times lower ( $0.12 \pm 0.02 \mu\text{W}/\text{cm}^2$ ,  $N = 17$ ,  $P < 0.001$ ).

## DISCUSSION

Males of *D. pumilio* and *D. auratus* appear to choose microhabitats with less UV-B, and are found in microsites within these microhabitats that are shielded from virtually all ambient UV-B radiation. Furthermore, UV-B levels at calling sites of *D. pumilio* are significantly less than expected at randomly selected locations in their natural habitat. These results, though correlative, suggest behavioral sensitivity of these two species to natural levels of ambient UV-B radiation in their environment. Other important factors are likely to determine microhabitat selection such as features that amplify mating calls or highlight the bright coloration of breeding adults as well as food availability and overall habitat quality. Predator avoidance is not likely to be a factor for these toxic frogs. Our results suggest that, in addition to these factors, dendrobatids may also account for ambient UV-B levels while choosing microsites.

Amphibians that are able to avoid even low levels of UV-B radiation may be sufficiently protected through behavioral avoidance as to render other energetically costly protection mechanisms unnecessary (production of photolyase, increased number of melanocytes in skin, increased pigmentation of embryos, *etc.*; discussed in Blaustein & Belden 2003). In light of current and projected increases in ambient UV-B, behavioral sensitivity could offer a selective advantage for some amphibians. The allocation of several protection mechanisms in some *Dendrobates* species (dark coloration, and behavioral sensitivity to UV-B) may indicate that UV-B was an important

selection pressure in the evolutionary history of this group, and that UV-B continues to exert strong selective forces on extant members.

Previous studies suggest that, even if direct mortality does not occur, various sublethal effects may impact amphibians exposed to UV-B. Among amphibian species that are vulnerable to damage by UV-B, negative effects are often specific to one life history stage and may affect different life history stages in different ways (Blaustein & Belden 2003). UV-B avoidance behavior may be particularly important for many *Dendrobates* species (including *D. pumilio*) that exhibit unusually prominent parental care by both males and females. Adults often transport developing tadpoles on their backs to suitable locations, and parents often continue feeding and monitoring offspring until metamorphosis (Savage 2002). Adults' sensitivity to UV-B levels may thus play an important indirect role in the survival of progeny through parental care. Interestingly, *D. pumilio* and *D. auratus* are two species for which there are no documented population declines, and *D. pumilio* are considered among the most abundant anuran species in the lowland forests of Costa Rica (Savage 2002).

How these animals are able to detect such low levels of ambient UV-B has not been examined. Detection of UV-B in these species may involve a higher than normal concentration of light-sensitive cones in the retina specific for ultraviolet wavelengths, as in other species (Fite 1976, Fite *et al.* 1998, Kelly & Bothwell 2002). Moreover, other methods of photoprotection of these species have not been quantified. The physiological mechanism for UV-B protection, detection, and behavioral avoidance present interesting areas for future research. It is worth further examining whether behavioral avoidance of UV-B radiation, alone or in conjunction with other defense mechanisms, plays a significant role in protecting other amphibian species against the biologically damaging effects of UV-B radiation.

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