

## Color polymorphism in *Leptodactylus fuscus* (Anura, Leptodactylidae): a defensive strategy against predators?

Sérgio Kakazu<sup>1</sup>, Luís Felipe Toledo<sup>2\*</sup> & Célio F. B. Haddad<sup>1</sup>

**Abstract.** Color patterns are strongly related to defensive strategies in anurans. Some anurans present more than one morphotype. *Leptodactylus fuscus*, for example, present two morphotypes (with and without vertebral white line). The proportion of each pattern in nature is different, whereby there are always more individuals without stripes. Therefore, we speculated if this difference in the observed color pattern is due to unequal predation pressures (i.e. stronger over the striped morphotype), and/or if there is a genetic component related to autosomic heritage. To test the selective predation over the morphotypes, we prepared plasticine models of *L. fuscus* with both phenotypes and placed them in the field. We did not find evidence of predation selection and as we found significant relationships between the proportions of the phenotypes and Mendelian proportions, we suggest that the phenotypes observed in this species are genetically determined (involving dominant and recessive alleles) and may not have a defensive function.

**Keywords.** Predation, coloration, defensive strategy, character heritage, evolution

### Introduction

Anurans are the basis of several food webs as they are preyed upon by several animal groups and even carnivorous plants (review in Toledo et al., 2007). They are largely consumed due to its high abundance in nature, relatively small size, and soft skin (Duellman and Trueb, 1986). Hence, several defensive strategies of which morphological and skin color adaptations are almost omnipresent have evolved to reduce the predation risk (see reviews in Hoffman and Blouin, 2000; Toledo and Haddad, 2009). Coloration may, besides other benefits, prevent the visually oriented predator to locate and identify its prey. In this case, anurans may have a benefit by means of polymorphism, which may vary among sexes, ages, and/or among individuals of the

same sex and age (Toledo and Haddad, 2009). The latter is present in the case of *Leptodactylus fuscus* (Schneider, 1799) showing two distinct color patterns (Figure 1).

Adult individuals of both sexes of *L. fuscus* may present a longitudinal white stripe in the vertebral region (Heyer, 1978; Figure 1). The proportion of occurrence of these phenotypes (vertebral stripe present or absent) in nature does not seem to be random since in a certain population there are always more individuals (generally from two to four times more) without the stripe than individuals with the stripe (Heyer, 1978). This fact leads us to rise two hypotheses: i) the absence of this stripe may protect individuals from visual orientated predators (such as birds and mammals), and therefore we can find more individuals of this phenotype in nature. In fact, there are some studies with lizards and snakes that indicate that the presence of longitudinal vertebral stripes are related to defensive mechanisms (e.g., Brattstrom, 1955; Sherbrooke, 2002); and ii) there may be a genetic component in this case that could be explained by the autosomic heritage of characters involving dominant and recessive alleles (Griffiths et al., 2006).

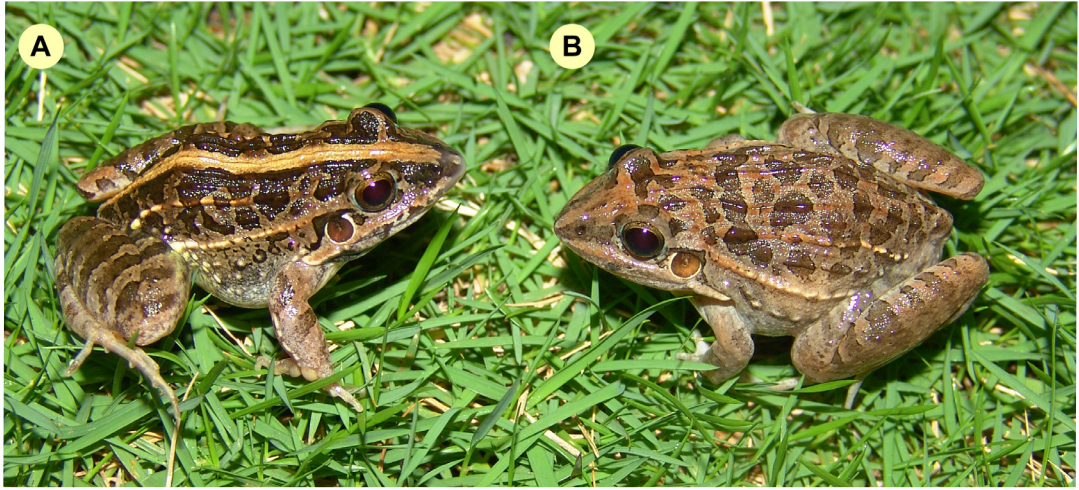
In the present study, we tested the hypothesis of selective predation over one of the phenotypes of *L. fuscus* in natural conditions.

---

1 Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), 13506-900 Rio Claro, São Paulo, Brazil;

2 Museu de Zoologia “Prof. Adão José Cardoso”, Instituto de Biologia, Universidade Estadual de Campinas, 13083-970, Campinas, São Paulo, Caixa Postal 6109; e-mail: toledof2@yahoo.com

\* corresponding author



**Figure 1.** Alive individuals of *Leptodactylus fuscus* with (A) and without (B) a vertebral stripe. Picture: Daniel Loebmann.

## Materials and Methods

To evaluate the proportion of phenotypes of *L. fuscus* in nature we collected data in two anuran collections respecting two criteria: i) all individuals of one collection and ii) individuals collected in the same site. In both cases only samples with more than 30 individuals/site or collections were considered. In total we were able to gather information of five groups: i) a population of the Floresta Estadual “Edmundo Navarro de Andrade”, municipality of Rio Claro, state of São Paulo; ii) a population originating from the Estação Ecológica de Itirapina, municipalities of Itirapina and Brotas, state of São Paulo; iii) a population of Campinas, São Paulo; iv) one sample with all the individuals of the Anuran collection Célio F. B. Haddad (CFBH; Unesp, Rio Claro, SP); and iv) one sample with all the individuals of the Museu de Zoologia “Prof. Adão José Cardoso” (ZUEC; Unicamp, Campinas, SP). These last two samples were used to test with a Chi Squared test ( $\chi^2$ ) (Zar, 1999) whether the proportion of the phenotypes is maintained independently of the population.

To test for the expected selective predation over the morphotypes, we prepared 300 models made of nontoxic gray, black, and white plasticine (Acrilex®) with both phenotypes of coloration (150 of each; Figure 2). To prepare the models we first created a counter mould of plaster made from a preserved individual of *L. fuscus*. This counter mould was covered with nail varnish (Colorama®) in order to cover air bubbles and other imperfections. We used surgical gloves to prepare the models in order to do prevent hu-

man smells that could attract or repel potential predators.

We placed these models in the field, in the Floresta Estadual “Edmundo Navarro de Andrade” (22°25'2.41”S, 47°31'18.93”W, 600 m a.s.l.), Rio Claro, São Paulo, Brazil, in the exact place where this species breeds during the breeding season (Toledo *et al.*, 2003). A total of 50 models comprising 25 models of each phenotype were placed randomly, by raffling the points which were interspaced by 1 m, into each of six plots of 100 m<sup>2</sup>. Experiments were conducted between 17 and 21 October 2005 (100 models) and 20 and 24 November 2006 (200 models), whereby the models remained in the field for five days. Plots were visited in the second day of the experiment to check whether there have been predation attempts upon the models. If we detected evidences, the corresponding models were removed from the plot in order to avoid double predation over the same model. For testing difference between the preyed rates over the two models we used a Chi Squared test ( $\chi^2$ );  $\alpha$  level was fixed at 0.05 (Zar, 1999).

## Results and discussion

Among the four groups of individuals tested, three groups (Rio Claro, Campinas, and ZUEC) were in agreement with the expected 3:1 Mendelian proportion (see Table 1). Data of one of the plots has been discharged, because the sunlight melted the models before the end

**Table 1.** Analyzed populations, number of individuals without and with a longitudinal stripe, proportion observed of individuals (without: with the dorsal stripe) for each population, and  $\chi^2$  for the proportion of 3:1.

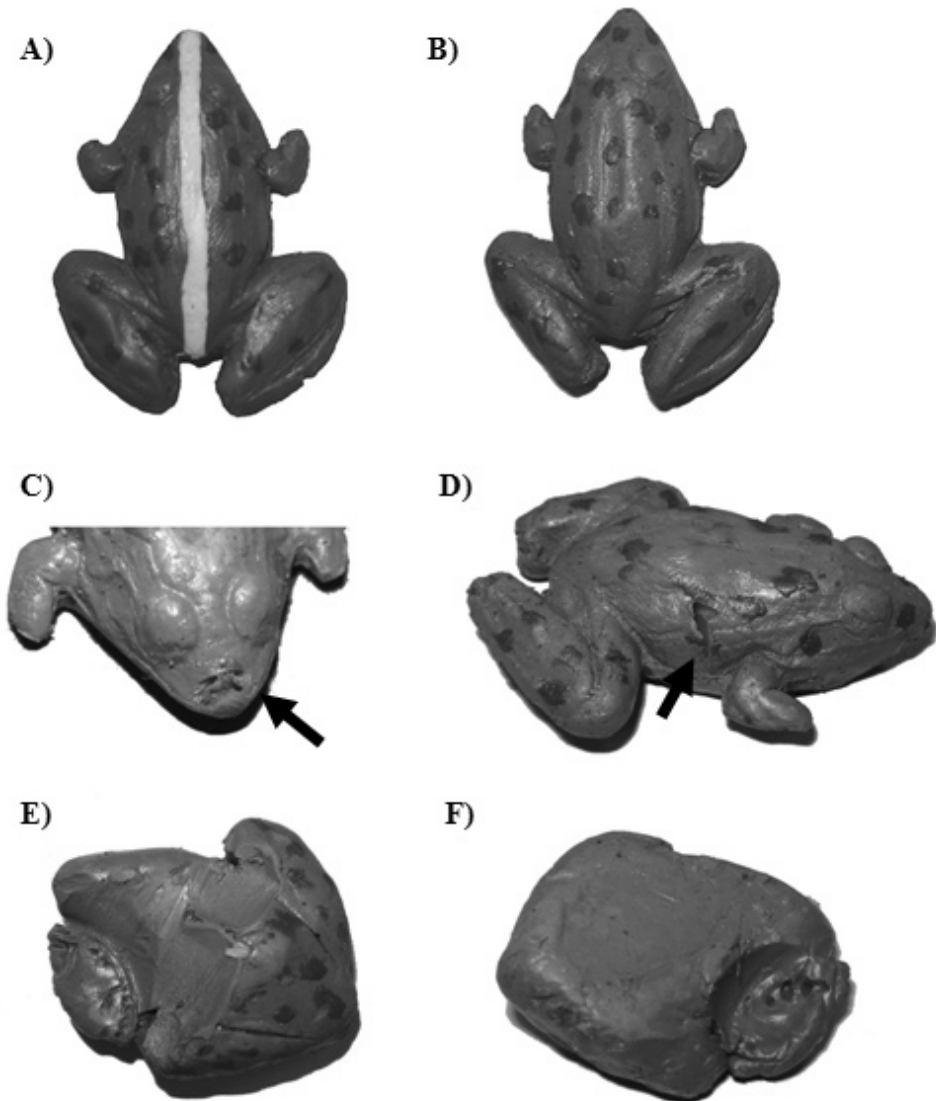
Site / collection	Without	With stripe	Proportion	$\chi^2$
Rio Claro, SP	28	07	4:1	$\chi^2 = 0.70; P > 0.05$
Itirapina, SP	127	63	2:1	$\chi^2 = 10.12; P < 0.05$
Campinas	24	8	3:1	$\chi^2 = 0.0; P > 0.05$
CFBH Collection	302	139	2.2:1	$\chi^2 = 14.99; P < 0.05$
ZUEC Collection	72	17	4.2:1	$\chi^2 = 2.48; P > 0.05$

of the experiment. Of the remaining 250 models placed in the field, 95 showed impressions which evidenced predation attempts and 20 were not found on its original place (Figure 2; Table 2). Therefore, 115 models (= 46%) suffered predator attacks. Among these, 57 had the white stripe and 58 did not. There was no difference between the predation rate over the two phenotypes ( $\chi^2 = 0.0086$ ;  $P > 0.05$ ). By analyzing the impressions made in the models we suspected that some of them were not from real predators of *L. fuscus* but most likely from

ants and other small insects. Therefore, after eliminating these records, we did another test, which also did not show a significant difference ( $\chi^2 = 0.38$ ;  $P > 0.05$ ).

Plasticine models have been used for amphibians to test the possible advantages of aposematic colorations (e.g., Kuchta, 2005; Saporito et al., 2007), but never for testing different cryptic morphotypes, as in the present study.

As we did not find evidence of predator selection (at least with immobile models we designed) and



**Figure 2.** Plasticine models of *Leptodactylus fuscus* with (A) and without (B) vertebral stripe, model that have been bitten in the nostril, indicated by an arrow (C), bird bite in the middle of the body, indicated by an arrow (D), and lizard bite in the head in dorsal and ventral views (E - F, respectively).

**Table 2.** Impression kind observed in the models, probable action that caused the impression, probable predator, and number of records registered.

Impression kind	Probable action	Possible	Records
Semicircular with sharp punctures	Bite	lizard or rodent	01
V-shaped perforation	Bite	Bird	11
Superficial scratches	Gnaw	Rodent	19
Incisive tooth-like perforation	Bite	Rodent	03
Sharp perforations in the head	Undetermined	Undetermined	06
Others	Undetermined	Undetermined	44
Not applied	Displaced	Vertebrate	30
Not applied	Overtumed	Vertebrate	01

as we found significant relationships between the proportions of the phenotypes of *L. fuscus* and of the Mendelian proportion, we are inclined to believe that the phenotypes observed in this species are genetically determined (involving dominant and recessive alleles) and does not have a defensive function, as previously indicated for species with color polymorphism (Toledo and Haddad, 2009).

**Acknowledgements.** We are grateful to Gabrielle D. Rosa and Cynthia P. A. Prado for helping during field activities; Daniel Loebmann provided the picture of individuals of *L. fuscus*. Dennis Rödder reviewed the manuscript; FAPESP and CNPq provided grants and scholarships.

## References

- Brattstrom, B.H. (1955): The coral snake mimic problem and protective coloration. *Evolution* **9**: 217–219.
- Duellman, W.E., Trueb L. (1986): *Biology of amphibians*. New York, McGraw-Hill Book Company.
- Griffiths, A.J.F., Wessler, S.R., Lewontin, R.C., Gelbart, W.M., Suzuki, D.T., Miler, J.H. (2006): *Introdução a Genética*. 8<sup>th</sup> ed. Rio de Janeiro, Guanabara Koogan.
- Heyer, R.W. (1978): Systematics of the *fuscus* group of the genus *Leptodactylus* (Amphibia, Leptodactylidae). *Nat. Hist. Mus. Los Angeles County Sci. Bull.* **29**: 1–85.
- Hoffman, E.A., Blouin, M.S. (2000): A review of colour and pattern polymorphisms in anurans. *Biol. J. Linn. Soc.* **70**: 633–665.
- Kuchta, S.R. (2005): Experimental support for aposematic coloration in the salamander *Ensatina eschscholtzii xanthoptica*: implications for mimicry of Pacific Newts. *Copeia* **2**: 265–271.
- Saporito, R.A., Zuercher, R., Roberts, M., Gerow, K.G., Donnelly, M.A. (2007): Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* **4**: 1006–1011.
- Sherbrooke, W.C. (2002): Do vertebral-line patterns in two horned lizards (*Phrynosoma* spp.) mimic plant-stem shadows and stem litter? *J. Arid Environ.* **50**: 109–120.
- Toledo, L.F., Haddad, C.F.B. (2009): Colors and some morphological traits as defensive mechanisms in anurans. *Int. J. Zool.* **910892**: 1-12.
- Toledo, L.F., Zina, J., Haddad, C.F.B. (2003): Distribuição temporal e espacial de uma comunidade de anfíbios anuros no município de Rio Claro, São Paulo, Brasil. *Holos Environ.* **3**: 197-212.
- Toledo, L.F., Silva, R.R., Haddad, C.F.B. (2007): Anurans as prey: an exploratory analysis and size relationships between predators and their prey. *J. Zool.* **271**: 170-177.
- Zar, J.H. (1999): *Biostatistical Analysis*. New Jersey, Prentice Hall.