Analysis of dorsal skin patterns as unique identifiers of *Xenopus laevis* frogs

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Abstract

African clawed frogs (*Xenopus laevis*) are a classic model organism, used worldwide by research laboratories to study developmental and cell biology, genetics, physiology, and immunology. Identification of individual research animals is important for both research productivity and overall animal well-being. Of the many methods used for tracking *Xenopus* animals, imaging of dorsal skin patterns is among the least invasive and inexpensive. We have implemented a curatorial system for identifying 126 frogs in a research facility. We confirm that individuals can be easily recognized by unique dorsal skin patterns, particularly when additional identifiable tank information is available. However, the long-term reliability of skin patterns is unclear. Whether or how much such skin patterns change over an individual's lifetime is not known. In order to assess the rate at which skin patterns change, images of mature adult individuals acquired over a three year timespan were compared. Skin patterns remained generally stable over this period with no major disruptions of existing patterns or emerging patterns occurring. Minor posterior shifting of the overall pattern was observed in some individuals, but patterns remained consistent nonetheless. We conclude that dorsal skin pattern imaging is a practical and highly effective method for identification of *Xenopus laevis*.

Introduction

Xenopus laevis frogs are widely used in scientific research since they are among the lowest phylogenic tetrapods, yet retain many of the conserved anatomical, physiological, developmental and cell biological characteristics of humans. Research labs that use *Xenopus* may have tens to hundreds or even thousands of frogs. Various methods have been historically used to identify and track frogs in captivity with mixed results. Ideally, the chosen method would provide a rapid and reliable way of identifying and tracking animals throughout their entire lifetime, while also being inexpensive and minimizing pain and distress to the animals (1). A very popular method to identify animals is the use of subcutaneous microchips, which is common practice in small domestic animals such as cats and dogs. While some labs do microchip frogs, this is one of the most expensive and invasive methods (2). After surgical insertion in frogs, microchips often move within the body and may even be excreted, making their permanence unreliable. Other methods of labeling animals, such as leg rings, tags, or tattoos as used on birds and cattle, are similarly not recommended methods for amphibians because of their delicate mucous-covered and highly vascularized skin (2). We examined here the utility of using unique dorsal skin patterns to distinguish individuals from one another. We tested whether frogs could be identified via simple photos over nearly a three year period in a habitat housing over 100 animals.

Methods

Xenopus laevis frogs were curated in a time frame of 3 months. Individual frogs were taken out of their tanks in the life support system, and photos were acquired of each of 126 individual animals. Additional identifying information including tank ID, sex and transgenic line ID were noted. Animals first photo identified in 2020 were compared to 2022 images to see whether gross skin pattern changes had occurred.

Results

Figure 1. Frog 'Q118', a wildtype female, was first imported to the life support system in February 2020. Images were acquired using three different devices over a 2½ year period.

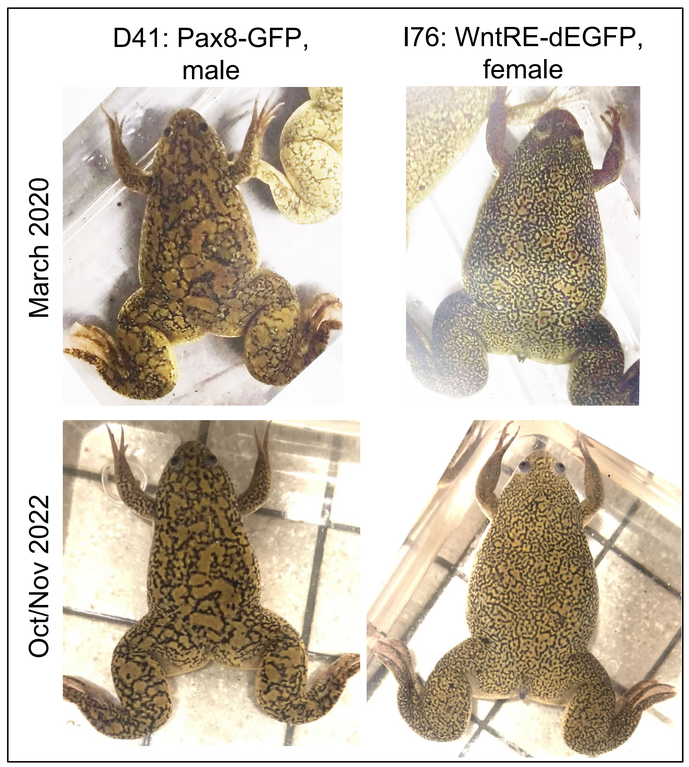

Figure 2. Images of two transgenic frogs first imported to the life support system in March 2020. 'D41' is a Pax-GFP male and 'I76' is a WntRE-dEGFP female. 2020 images were acquired at the National Xenopus Resource in Woods Hole, MA prior to transfer. Despite differences in age, means and location of image acquisition, and color tones, sufficient distinctive skin markings were maintained over time permitting the clear identification of these individuals.

Future Directions

- Curate frogs that were under a year old in 2022 and compare to images that will be taken late 2023
- Examine whether their patterns changed significantly by either skin patterns growing, moving, or new patterns emerging
- Development of electronic scanners/AI-based pattern-matching for detecting marking patterns could increase potential of this method

Figure 3. Pictures acquired in November 2022 of young *Xenopus laevis* hatched June 2022 (5 months old).

Conclusion

- There were no dramatic changes in the skin pattern of *Xenopus laevis* that precluded the ability to accurately identify individuals.
- Patterns in very young or albino individuals are not as easy to see.
- Dorsal skin markings appear to remain consistent but relative skin color can alter as a result of stress or fluctuations in the environmental lighting.
- Although The Guide allows for generalized approaches to frog accounting (1), photo-based identification is a practical, affordable and reliable method for the tracking of individual frogs.

References

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