The Chromosomes of Terraranan Frogs Insights into Vertebrate Cytogenetics

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A piece of amber (15–20 Mya) found in the La Toca amber mine in the Dominican Republic on Hispaniola containing a nearly complete fossil *Eleutherodactylus*. It represents the first amphibian fossil in amber and the oldest amphibian fossil from Mesoamerica (see Poinar and Cannatella, 1987). Photography generously provided by George O. Poinar, Jr. (Department of Zoology, Oregon State University, Corvallis, Oreg., USA).

The Chromosomes of Terraranan Frogs

Insights into Vertebrate Cytogenetics

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Foreword

Nearly half a century has passed since I collected tissues and, with the assistance of a graduate student, squashed and stained anuran testes to determine the chromosome numbers of a small number species. The two publications that resulted joined a meager number of others that now are viewed as primitive attempts to understand the significance of chromosome number and structure in vertebrate organisms. Since then, like many other biological disciplines, cytogenetics has matured from its infancy into a highly sophisticated field that challenges researchers in the development of new techniques and broader taxon sampling to interpret the relevant evolutionary processes more effectively.

One of the most prominent leaders in this rapidly developing discipline is Michael Schmid, in whose laboratories in the Department of Human Genetics at the University of Würzburg in Germany, many of the cytogenetic techniques have been initiated, refined, and subsequently disseminated in multitudinous publications. Michael joined forces with another pioneer of amphibian cytogenetics, James P. Bogart, and then with S. Blair Hedges, in an international effort to describe the karyotypes and interpret the cytogenetics of *Eleutherodactylus* and related anurans, now grouped in Terrarana. The resulting *The Chromosomes of Terraranan Frogs – Insights into Vertebrate Cytogenetics* has seven additional collaborators from four other countries: Costa Rica, Cuba, Venezuela, and Taiwan.

One might reasonably ask how data on the chromosomes of a group of frogs can occupy an entire book? Or, what is the relevance of data on terraranans to our understanding of chromosomal evolution? The answers to these questions are explicitly obvious in this book.

Terraranans are an immense group of frogs. This monograph is the most comprehensive, original and comparative cytogenetic study of vertebrates, but it accounts for only 261 (28.2%) of the recognized species of terraranans. Nevertheless, the results are interpreted in the context of published cytogenetic data for other amphibians as well as for other vertebrates. The volume is organized in such a way that general chapters that may be useful to non-cytogeneticists pre-

cede the specialized sections on Terrarana. The structures of terraranan chromosomes are described in exquisite detail, based on observations of a variety of staining and banding preparations as well as in situ hybridizations. The illustrations of chromosome structure and modifications are extraordinary and presented in a manner that is useful for taxonomic comparisons.

The evolution of karyotypes of terraranan frogs is determined by mapping the karyotype characters of the examined terraranan species on the molecular phylogeny provided by Hedges, Duellman and Heinicke in 2008, to determine the most parsimonious arrangements. The calculations yield a frequency of 0.40 changes in chromosome number per Myr of divergence between two lineages. This equals one mutation of chromosome number every 2.5 Myr and compares favorably with the average rate of 0.4 to 1.4 chromosome rearrangements per lineage per Myr for the most rapidly evolving groups of mammals. Thus, terraranans have the highest rate of chromosomal mutations known in amphibians.

This prolific monograph clearly demonstrates that terraranan karyotypes do not conform to the generally accepted maxim of ‘conservative chromosome evolution’ in amphibians that derives from the observation that species in most amphibian taxa share the same, or nearly the same, karyomorph.

Insofar as known, all terraranans, except the ovoviviparous *Eleutherodactylus jasperi*, have the same reproductive mode; moreover, they are quite conservative morphologically within lineages. Perhaps the forthcoming generation of evolutionary biologists will examine the karyotypes of many more species of terraranans and include still more species in the molecular phylogeny. Then they may be able to address the compelling question: Why do terraranans have such great variation in their karyotypes and such high frequencies of mutations?

William E. Duellman
Lawrence, Kans.
August 2010

Preface

The very first expeditions to the Neotropics and chromosome analyses for the present monograph on Terrarana were realized by James Bogart over 40 years ago in 1967. Later, in the early 1980's, James Bogart and Blair Hedges initiated a long-term collaboration on Terrarana, then mostly called *Eleutherodactylus*, primarily from Caribbean islands. In 1987, Michael Schmid and his group started to perform collecting trips in Central- and South America as well as in the Caribbean region. In 2001 we decided to share all the cytogenetic data obtained from many terraranan species and aspired to engage in a large comprehensive study on the evolution and structure of terraranan karyotypes. Finally, in 2005, Shyh-Hwang Chen accepted our invitation to participate in this project and contributed his results on Central American terraranans.

The one thing that has hampered research on these animals, including chromosome studies, has been a poor understanding of their evolutionary history, in turn reflected in their taxonomy. This was through no fault of systematics, because terraranans apparently have very few diagnostic morphological traits (Lynch and Duellman, 1997). Lynch (1986) discovered one such character, which correctly defined a group of terraranans (*Craugastor*) that contains about 10% of the species, mostly in Central America. Molecular studies were more fruitful, confirming Lynch's finding and identifying other major clades of the terraranan evolutionary tree (Hedges, 1989a; Hass and Hedges, 1991; Crawford and Smith, 2005; Heinicke et al., 2007, 2009; Hedges et al., 2008a). Undoubtedly more major clades will be found, and some branches of the current tree revised. Separate from recognizing an evolutionary tree is deciding what to name the clades (taxonomy). One of us (S.B.H.), and colleagues, recently proposed a major revision of the taxonomy (Hedges et al., 2008a), including the introduction of the name 'Terrarana', which is followed in this monograph. While we believe that this new taxonomy has finally pro-

vided much-needed stability, which will facilitate future research, we fully expect further revisions in the future as new species and clades are discovered.

When, in 2001, we decided to collaborate on launching this book, we were far away from realizing how many efforts were still necessary to reach a stage where all participants were content with the final manuscript draft and the hundreds of illustrations. We (Michael Schmid, James Bogart and Blair Hedges) have met five times at Pennsylvania State University (2004), at the University of Guelph (2004, 2009), and at the University of Würzburg (2001, 2008) for in-depth discussions. We exchanged endless electronic messages. Several large parcels containing photographic negatives or huge files with karyotype illustrations made transatlantic voyages by courier. In Würzburg, where the data were accumulated, Michael Schmid and Claus Steinlein were often overwhelmed with data and, during some moments, were unsure whether the monograph could even be completed. The diversity and complexity of terraranan karyotypes, which is the result of an extraordinary fast chromosome evolution in this group of amphibians, challenged all cytogenetic knowledge and made it necessary to consider large parts of the previous cytogenetic and herpetological literature.

The present monograph represents the most comprehensive original comparative cytogenetic study on vertebrates, or even eukaryotes. It comprises 261 different recognized species of Terrarana. Furthermore, the results obtained have always been interpreted in context with the cytogenetic data published for other amphibian taxa or for other vertebrates, invertebrates or even plant species. The general properties of eukaryote chromosomes, the rules governing structural and numerical chromosome repatterning, as well as the principles of chromosome evolution are explored. The extensive section of 'Material and Methods' was compiled with the intention to provide the necessary meth-

odological guidance for those who are interested in studying amphibian chromosomes. We consider this monograph to be useful for providing detailed and archival information on terraranan karyotypes and genomes to our colleagues who are already recognized specialists in amphibian or vertebrate cytogenetics. We also expect that this monograph will provide comprehensive insights into vertebrate cytogenetics for students.

Notwithstanding the large volume of data presented here, we acknowledge that many questions concerning chromosome evolution in terraranan frogs remain unanswered. Also, we are aware that some views presented here

are likely to change in light of further work, including that resulting from newly-developed molecular cytogenetic techniques. Nonetheless, it is our hope that the wealth of information presented here will provide a foundation for, and stimulate, future research on these remarkable frogs and on chromosome evolution in general.

Michael Schmid, Würzburg
James P. Bogart, Guelph, Ont.
S. Blair Hedges, University Park, Pa.
August 2010

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In a project of such magnitude as the present monograph on terraranan cytogenetics, which was carried out over a period of 38 years (1967–2004), on more than 70 expeditions to the Neotropics and in several laboratories, we asked for and received the support of many colleagues and friends. The Editors of the monograph (Michael Schmid, James P. Bogart and S. Blair Hedges) acknowledge with great pleasure all the persons mentioned in the following and thank them for their invaluable help.

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Remarks

Remarks on the use of scientific names

Throughout the present monograph the scientific Latin names used for the species of *Terrarana* strictly follow the systematics and nomenclature of Crawford and Smith (2005), Heinicke et al. (2007, 2009), Hedges et al. (2008a, b) and Padial et al. (2009). Common names of the terraranans have been avoided.

The scientific names of all other amphibian species referred to in the text and tables are the same as were used in the cited original publications. Recent changes of scientific names of non-terraranan amphibians are provided in Appendix V and are regularly updated in the electronic database of Frost (2010). Occasionally, the common names of some non-terraranans are added to the scientific names.

The scientific names used for all non-amphibian species (vertebrates, invertebrates and plants) referred to in the text are the same as in the original cited publications.

Remarks on the illustrations, tables and appendices

The legends to the figures 1–52, 2045, 2046, 2066 and 2067 are placed beneath or on the page before or behind the corresponding illustrations. The legend and all explanations to the figures 53–2044 are found in chapter 3.59. The color figures 2047–2065 showing the living terraranan species have no legends and are not referred to in the text. These figures are placed at the end of the monograph.

The tables 1–18 are distributed within the text of chapters 2 and 3 and, in most cases, are placed next to the pages where they are referred to.

The Appendices I–V, which provide supplementary information, are found at the end of the monograph.