Citizen Herpetology

Part 2—Some of the Questions that Molecules Can Answer

by Dustin Rhoads

This spring, *Cosmos: A Spacetime Odyssey*, a reboot of the classic series written by astronomer Carl Sagan, debuted on several major television networks, including Fox, NatGeo, and their affiliates. The second episode concerned itself with the topic of evolution and was titled, "Some of the Things that Molecules Do," which was, in fact, part of a line Sagan spoke in the original series. Well, the subtitle of my first installment in this series was a "shout out" to a chapter written by a popularizer of science, and so in keeping with that spirit of honoring the great educators of the masses, consider the title of this article a "shout out to a shout out." Follow that? Okay, good…let's carry on, then.

One of the things my graduate school advisor taught in 2012 in a class called Molecular Laboratory Techniques was that, in the near future, when someone finds a specimen they have trouble identifying to, say, the level of species, instead of scouring through field guides, all they will have to do is get a tissue sample. DNA sequencing will, by then, be so cheap and common that it will be the standard for laypersons and professionals alike.

Just muse for a moment on the implications of how the aforementioned technology will affect naturalists. It should leave many of us with an urgent feeling—a need to "run to catch up." My goal in this and the next installment or two is, thus, to bring us all up to speed on some of the available tools and types of questions that can be answered as natural history merges with molecular technology. Undoubtedly, much of what I remember from graduate school from less than two years ago has already seen *several* facelifts (e.g. newer, better software and so forth), so I will try my best to make this all relevant.

As I have stated in Part 1 (Rhoads 2013), herp enthusiasts are a group perfectly tailored to discover a particular set of biological phenomena. For instance, since many are involved in selective breeding of captives, they are the group most likely to discover rare genes that alter some aspect of anatomy, especially pattern and pigmentation. Such mutations are extremely important to the survival of reptiles and amphibians in nature (Norris and Lowe 1964) and—quite arguably—in captivity. Let us consider some examples that have occurred to me that I either (a) have started to pursue or (b) wish to pursue. You might even consider this an informal research proposal. (Or *grant* proposal, anyone? I jest—kind of).

On the Origin of Scales

One of the ways of experimentally discovering whether, and how, a feature is an adaptation is by removing the use of the feature in a "treatment group" and seeing if performance is altered between the treated and untreated groups (the next step would be to see if there is a difference in survival and reproductive fitness between the two groups). Someone who wants to measure the usefulness of having opposable thumbs in primates might, for instance, subject several groups of primates, including humans, to tape their thumbs down to their palms and see how proficient they are at manipulating objects with their hands sans thumbs. I can imagine that for many a long year, scientific naturalists have wished they could render a reptile stark scaleless so they could test questions related to adaptation concerning scales.

Well, it turns out that just four decades ago, some scientists finally got that chance. A single scaleless Pacific Gopher Snake (*Pituophis catenifer catenifer*) was found near Oakland, California. The researchers investigated hypotheses concerning water loss and heat transfer, and though their sample size was as small as possible (one individual), the results allowed them to conclude with at least some confidence that any difference in performance of these two physiological functions was negligible. They suggested scales probably held other adaptive uses such as mechanical protection (Licht and Bennett 1972). (One can imagine the usefulness of a scale-covered body in harsh terrain. I recently told a group of fifth-grade science students to imagine having fingernails covering their entire body, which of course, evoked many a drawn out "Eeeeeeewwww!" from some. I then asked them to imagine whether it would hurt if they rolled over broken glass or bumped into cactus spines – nearly all who answered said *no*.) There are many other potential uses, so it's clear more work needs to be done to determine the role of scales as adaptations.

But what about the *gene* responsible for scalelessness? How would you find it inside that enormous genome? How would doing so enable you to shed light on the evolutionary history of scales in *all* reptiles? Let us answer these questions, one by one.

First off, we know from several collective sources that, so far, all scaleless snakes of species in the colubrid family carry an autosomal, recessive gene, which causes the homozygotes to have reduced or missing scales on the dorsal aspect of their bodies, while similar mutations in Ball Pythons (*Python regius*) and Bearded Dragons (*Pogona vitticeps*) carry an autosomal, co-dominant or incomplete dominant gene.

A genetic mutation in a Ball Python (*Python regius*)—scalelessness. Photo by Brian Barczyk, BHB Reptiles; used with permission.

But, again, how would you go about finding the gene? When searching for genes, it's helpful if you have an entire sequenced genome to work with, and none of these species have their entire genomes sequenced. But it's also helpful if you can try candidate genes that have been found in other taxa, however distantly related. So, what to do then?

I started thinking about what I knew regarding reptile scales. I knew they were made of the same stuff bird feathers are made of, namely beta (β) keratin. I further knew that (phylogenetically) birds are considered *avian reptiles*, and are themselves more closely related to crocodilians than either are to other groups. Consequently, a likely hypothesis seemed to me that bird feathers and reptile scales were *homologous*—that is to say, inherited from a common ancestor, with modifications (e.g. A seal's flipper, a bat's wing, and a human arm are all homologous, while in contrast, a Thorny Devil's [*Moloch horridus*] thorns and propensity to consume ants are *analogous* to similar traits in North American horned lizards of the genus *Phrynosoma*.)

So, I wondered, are there any lineages of birds that are also scaleless? As I searched, it turns out there are! It is a mutation in chickens. The gene is even called "*sc*" for scaleless, and the homozygous birds have zero scales and are nearly featherless as well. But alas, among their closest living relatives, only the Jungle Fowl's (*Gallus gallus*) genome was sequenced at the time, and the *sc* gene had not yet been mapped (found).

However, in early 2013, I gave the chicken *sc* gene yet another internet search to see if there were any new discoveries, and a team of researchers had indeed just mapped it (Wells *et al.* 2012). The mutation was found in *FGF20* of chromosome 4, a region that also codes for hair follicle growth in mammals. This was exciting, but I was also entering unfamiliar territory at this point.

Yep, your eyes aren't lying. That's a living, breathing featherless and scaleless chicken (*Gallus* sp.), and not much worse for the wear either. Photo by Adi Nes, Jack Shainman Gallery, NY; used with permission.

From my rather limited experience working in laboratories, I knew I needed to use the location of the newly found chicken *sc* gene to somehow find a homologous gene in a scaly reptile species that has a scaleless morph. In other words, I knew I needed to locate the *FGF20* gene in a lizard or snake species with known scaleless mutants among its conspecifics. I decided my species of choice would be the Texas Ratsnake (*Pantherophis obsoletus*). I chose them because I knew this species is among the most commonly-available scaleless snake species in captivity, and it would be easy to get blood tissue samples from a nearby breeder.

Finding the scaleless gene in these snakes still seemed tricky. After all, the genome for the Texas Ratsnake had not been sequenced. First I contacted a herper friend at Cornell University's Lab of Ornithology. As coolly as if she was chatting with a "BFF" about where to get her nails done, she suggested I perform what is called a "BLAST," then do a "sequence alignment," and then "create oligonucleotide primers," and that would get me started in the right direction, she said.

Uh-huh. My first thought was how lucky I am to have a friend who is a freaking genius. My second thought was I needed to find a translator for whatever she just said. Okay, to be fair to myself, I vaguely remembered BLAST, and I had some experience with alignments and primers, but suffice it to say my molecular biology was a little rusty. In any case, I could not remember how to do any of them without some guidance. This was when I consulted one of the most helpful investments I have ever purchased, a book called *Reading the Story in DNA: A Beginner's Guide to Molecular Evolution*. I emphatically want to stress that I cannot overstate the usefulness of this totally approachable book to people like myself, people who are interested in whole organisms who simply want to use the often difficult-to-grasp, abstract concept of molecular technology to ask questions about their favorite creatures. This book was written with exactly that audience in mind, and the care and concern for the reader is evident everywhere. (I should also add: I have been in recent contact with the author, and she is preparing a second edition of the book, one with several new chapters. The first edition is only just out of print and hard copies are already prohibitively expensive. A Kindle version is still available, however, and is very reasonably priced, in my opinion.)

BLAST is basically an online database search to find DNA sequences that closely match the queried sequence. For instance, if you look up a particular mitochondrial gene sequence for *Varanus komodoensis*, the Komodo Dragon, and then you BLAST it, it will bring up a list of the closest matches to that sequence, which will likely come from other varanid lizards, given their close evolutionary relationships. It will also show you the percentage similarity of each sequence to the original query. The database where you can look up any sequence of any organism—which is also the database BLAST searches—is called GenBank (http://www.ncbi.nlm.nih.gov/genbank/); it's a sort of "Wikipedia" for molecular sequences where anyone can upload or download sequence data. I would suggest playing with it, because it's very fun *and* interesting to see for yourself the close relationships between humans and chimpanzees. There are YouTube videos that walk you through the steps of a BLAST search.

Now remember, if I were to perform a BLAST search using the chicken *sc* (or *FGF20*) gene, it would not pull up a Texas Ratsnake homologue of that gene, since that work has not yet been done for Texas Ratsnakes. But it *might* pull up homologues for species that are closer relatives of the Texas Ratsnake than are chickens, and that is the goal. In my query, I found the *FGF20* homologue for *Anolis carolinensis*, a scaly reptile species whose genome has been sequenced.

I could then use that *Anolis* sequence and align it with that of the chicken *FGF20* homologue. *Alignment* is the process of arranging multiple DNA sequences so that all of the homologous (commonly inherited) sites are lined up in columns. When you properly align sequences from a number of close relatives, along with a number of individuals from an unrelated *outgroup*, it becomes obvious which parts are likely homologous and which parts are not. (A great, brand new tutorial using anoles can be found here: http://www.hhmi.org/biointeractive/using-dna-explore-lizard-phylogeny. There are also helpful how-to videos on YouTube for creating proper alignments, as well.)

From that alignment of chicken and *Anolis* DNA, I could then develop *primers* or short sequences of DNA that are specific to the *FGF20* gene in squamates. Once I extracted DNA from Texas ratsnake blood, these primers would then enable me to make lots of copies of the *FGF20* gene of Texas Ratsnakes in the laboratory. This process of making lots of copies of a target gene or strand of DNA is called *DNA amplification*, and it uses a technique called *polymerase chain reaction* (PCR). As alluded to earlier, I acquired blood tissue from scaleless, heterozygous-for-scaleless, and wild-caught ("wild-type" and, presumably, non-heterozygous for scaleless) Texas Ratsakes. For molecular biologists, the useful thing about reptile blood, unlike mammal blood, is that each red blood cell has a nucleus, inside which is an entire copy of the organism's genome. Using PCR, I can then make (or *amplify*) many copies of the *FGF20* sequence from each of the three abovementioned genotypes of Texas Ratsnake.

From there, I can *sequence* the DNA of the *FGF20* gene in the three ratsnake genotypes and see if there is a mutational difference between them in that gene. Doing so would answer the question of whether the same gene that causes scalelessness in birds also causes scalelessness in snakes.

What would be the steps to answer this question?
• BLAST the chicken FGF20 sequence.
• From that BLASTed list, use a snake (or lizard) genome to align the *FGF20* sequence with that of the chicken (*Gallus* sp.).
• Create squamate-specific oligonucleotide primers from the alignments.
• Get tissue samples from wild-type, heterozygous, and homozygous scaleless snakes.
• Extract the DNA.
• Choose PCR thermal cycling reaction.
• Run the PCR.
• Sequence.

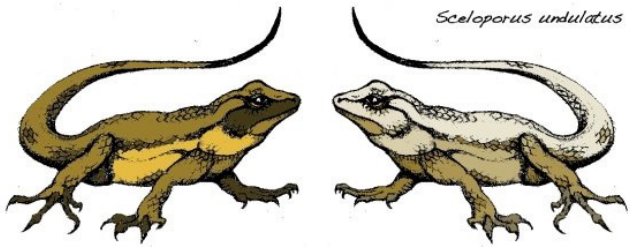
Is this question *important*? Who knows? But I've learned to not worry about that. As the author of the book I recommended above so self-affirmingly said,

> Now the thing to do is get out there and get your hands dirty: think of a question and work out how you could answer it, contrast hypotheses and think of how you would test them, delve into the data and see what you find. Do not fuss about whether your question is too big or too small, as long as it is a question you find interesting, and you think you can see a way of shedding light on it. The only way to start is begin with something, anything, and see where it leads you (Bromham 2008).

Even while researching this topic, I have found other kindred spirits who have mused on the likely significance of studying this question. One set of authors stated that "Understanding the origin and evolution of the β keratin gene families in reptiles and birds will undoubtedly add to our understanding of the evolution of skin appendages such as scales and feathers" (Sawyer, Glenn, and French 2000).

Evolutionary genetics has already solved one of the oldest and most famous of riddles, "Which came first—the chicken or the egg?" (i.e. phylogenetic trees of tetrapods show eggs clearly existed long before chickens). If nothing else, maybe pursuing this problem will one day help answer the age-old, fable-like mystery of "How did the snake get its scales?"

A Possible Research Gold Mine



Evolution of the *mc1r* gene in *Sceloporus undulatus* at White Sands, New Mexico. Illustration by Simone Des Roches; used with permission.

Every now and then, a single discovery can open up a seam, from which outpours a veritable geyser of research productivity. The discovery of the melanocortin 1 receptor (*mc1r*) gene, for instance, has allowed many careers and laboratories to materialize in its wake. Off the top of my head, Erica Rosenblum (UC Berkeley), Hopi Hoekstra (Harvard), and Michael Nachman (UC Berkeley) come to mind as prominent researchers who perhaps owe much of their success as scientists to investigating this gene. Studying natural selection at this locus, or location in the genome, has become a poster child for public outreach regarding observed instances of microevolution (population genetics) leading to macroevolution (speciation). From blanched and melanistic populations of lizards and mice on gypsum dunes and lava fields in New Mexico and Arizona, to white-phase "spirit bears" in British Columbia, and even quite possibly to different color morphs of woolly mammoths now buried in Siberian permafrost, research of what variation in this gene does to melanin pigment and speciation has been remarkable and applicable in a wide range of phenomena

and organisms. Examples of such have been in textbooks and presented in television documentaries such as PBS's *What Darwin Never Knew* (2009) and the most recent (2014) version of *Cosmos* series mentioned previously. And while it has shed light on understanding color variation in many vertebrate populations, especially mammals, nothing is known of the genomic whereabouts of the largely unique-to-herp skin color pigments—red, yellow, and orange—the xanthophores. What a world of discovery this would open up for a new generation of herpetologists!

As in the scaleless squamate example discussed earlier, to find the locus or loci that govern xanthophore expression, it would help to use (a) a taxon whose genome has been entirely sequenced and (b) a taxon with known individuals lacking the feature in question (i.e. the xanthophores). In this case, we have both.

Anolis carolinensis has both a sequenced genome and *axanthic* (lacking yellow pigment) individuals. Since they are typically green when normally pigmented, axanthic specimens are a stunning blue coloration—but there's a catch. They're quite rare. As stated by one source:

> Occasionally, pastel blue specimens of green anoles are collected and offered in the trade. These blue anoles . . . are few and far between. According to one anole distributor in Louisiana, there is one blue anole brought in by collectors per at least twenty thousand specimens. With some efforts in selective breeding, more specimens could become available in the future. As could be expected, these blue anoles fetch high prices, often $100 or more at the retail level (de Vosjoli 2012).

Green minus yellow = blue. Shown are an axanthic female and normally pigmented male *Anolis carolinensis* in the throes of passion. Photo by Maurice Pudio II; used with permission.

This is still the case. In fact, I have found one person who has a very small number of them and has produced one or two, but he has not offered any for sale yet, and he has them priced at about $400 (US) each. I have also tried, off and on, for nearly 10 years to buy one from a reptile distributor who claims to have blue phase anoles breeding on his grounds and used to have them on his pricelist at around $100. "Anolologist" Jonathan Losos (pers. comm.) has also endeavored to find and acquire some. No luck so far.

The difference between tackling this potential study and the scaleless one would be the fact that there's no candidate gene known in other taxa, which would also mean that a respectable number of blue individuals would be required to find a statistically likely genomic site, e.g. a distinct nucleotide difference between mutants and nonmutants. A group would, of course, be prohibitively expensive to purchase (if groups of them were even available, which they are not). Therefore, buying one or two and breeding them would be the most cost-effective and most plausible option, and even ideal, so that a family tree of "affected" and "unaffected" individuals could be both known and sampled.

Given the rarity and apparent lack of incentive for establishing blue phase green anoles in captivity, I have thought of at least one fallback organism to test this question with—the sequencing of the genome of the Common Garter Snake (*Thamnophis sirtalis*) is underway and possibly even nearing completion (Castoe *et al.* 2011), and axanthic individuals of the red-sided variety are both inexpensive (usually under $50) and well-established in captivity. I hope to procure a "study group" of homozygous, heterozygous, and wild-type individuals so that a genealogy can be established and documented and their tissue samples catalogued. Another potential fallback organism would be the Corn Snake (*Pantherophis guttatus*), as there have been proposals submitted to sequence the genome of this taxon at Bangor University in the UK (Castoe *et al.* 2012), and axanthic (Anerythristic Types A and B) individuals are both inexpensive and broadly established in captivity.

To actually find and map the mutation, something like an *association study* using a *hypothesis-free genome-wide scan* would be needed. Association studies are where informative pedigrees are used to identify regions of the genome that segregate with the mutation, and doing a hypothesis-free genome-wide scan means looking for genetic markers significantly associated with the mutation throughout the entire genome, rather than targeting suspected candidate genes. Another possible way to investigate this question could be to work backwards. That is, find the xanthic and axanthic pigments and analyze their proteins first, then figure out the exact amino acid sequence that make those proteins, and finally the nucleic acid sequences that translate into those amino acid chains.

As you may have already well intuited, genome-wide scans are the method often used to find genes associated with inherited diseases in humans. It's also the method that was used to find the *sc* gene in chickens.

One interesting fact about axanthism: Unlike many other highly dramatic, lack-of-function mutations affecting skin pigments (such as amelanistic albinism), axanthism (also called *anerythrism*, when

referring to red xanthophores), on the other hand, has actually come much closer to approaching fixation in some populations. (*Fixation* means all members of the population carry the same allele at a particular locus and there is no polymorphism at that locus.) There are black and white axanthic Eastern Mud Snakes (*Farancia abacura abacura*) in southeastern North Carolina, and in one study, 8% of well over one hundred specimens observed were homozygous mutants (Bechtel 1995). Assuming recessive inheritance and that these numbers represent real populations and the real frequency of alleles (which I concede is assuming a lot), when I apply a basic population genetics equation ($p + q = 1$), it calculates nearly half of all individual mud snakes in the population would be at least carriers of the axanthic allele. To boot, axanthic Corn Snakes are rather frequently found in Florida near Lake Okeechobee south to Homestead (Bechtel 1995; R. D. Bartlett, pers. comm.). And while these examples of possible incipient fixation are fascinating, there are likely many populations, subspecies, and full species whose members have all become entirely axanthic or even *hyperxanthic*—'desert phase' California Kingsnakes (*Lampropeltis getula californiae*) and Isla Gorgona Blue Anoles (*Anolis gorgonae*) readily come to mind as potential examples of the former.

Two possible examples of axanthism gone to fixation in the wild? Desert phase California Kingsnake (*Lampropeltis getula californiae*) and Isla Gorgona Blue Anole (*Anolis gorgonae*). Photos by Troy Hibbitts (l) and Matthias Jurczyk (r); used with permission.

Since innumerable mutations affecting xanthophore expression are documented in a wide array of herp species and naturally occurring lineages, it stands to reason that finding a locus for one such mutation in a single taxon might serve as a candidate gene for other species and would open a highly productive field of study for population geneticists and evolutionary biologists who study herps.

Using Other People's Data to Answer Your Questions

Part of the genius of GenBank is that many people can make discoveries to scientific questions without ever setting foot in a laboratory. The molecular, amino acid, and protein data that is available there can be accessed and used by anyone in an infinite number of ways to test hypotheses to interesting puzzlements in nature.

A striking example of sourcing such data for a study and later turning it into a high-profile publication can be found in a 2011 issue of the prestigious journal *Evolution*. John Wiens at Stony Brook University downloaded genetic data from GenBank for 170 different species of amphibians to create a time-calibrated phylogeny that showed how long ago teeth on the lower jaw of frogs were lost (~230 mya)—and subsequently and much more recently (~5-17 mya)—regained. Not only that, but this study has also turned a long-recognized fact of evolutionary biology, namely Dollo's law, on its ear (Wiens 2011).

I have myself often marveled on the curious defensive behaviors of North American snakes in the xenodontine clade in the subfamily Carphophiinae—especially the hog-nosed snakes (*Heterodon* spp.), ring-necked snakes (*Diadophis* spp.), and Mud Snakes (*Farancia abacura*). All of them exhibit an array of interesting ethology such as fainting or death feigning, ranivory (or other amphibian diet), hissing, neck-flattening, tail-curling, tail-poking, and belly-exposing of bright ventral colors. What, and when, were the evolutionary steps of developing such behaviors in this group throughout space and time?

Cave-hanging for bats is well-documented in several snake (and even a centipede) species. Some of these snakes are very distantly related; others, more closely so. At least one taxon hangs from the ceiling of the cave with its mouth wide agape for the duration of this waiting game. How many times has this behavior evolved in snakes? In which major lineages has this behavior likely *never* arisen?

Hunter and hunted—which is which? Cave explorer Arturo Bayona Miramontes (la Universidad Autónoma de Guadalajara, Jalisco) observes a cave-hanger swallowing a microchiropteran at La Cueva de las Serpientes Colgantes (literally, "the Cave of the Hanging Snakes") de Kantemó, Quintana Roo, México. The snake, *Pseudelaphe phaescens*, dangles the anterior half of its body from the limestone ceilings of bat dwellings until one or more of the flying mammals find themselves in the wrong place at the wrong time. Photo by Alberto Friccione; used with permission.

Both the xenodontine and cave-hanging questions above lend themselves well to studies from which all genetic data needed could be found already uploaded to GenBank by other researchers.

The Secret Lives of Reptiles

If there's any book that still needs to be written about reptiles, it would have to be one that follows the lives of several individuals on a daily basis from the day they are conceived until at least their

first reproductive event. It's too bad we lack the around-the-clock staff, ingenuity, or scientific instruments for such a task. Hopefully, we humans can survive our own technological adolescence without destroying ourselves, and other earthlings such as snakes, long enough to accomplish such feats of natural history study. Until then, we have to use other methods to peer into the stubborn secrecy of Mother Nature.

One such method is molecular biology. We've already alluded to the fact that individuals can be identified to taxonomic levels via these methods. Even if you were to find just a fragmentary piece of shed skin, you could still put a name to it, using a PCR machine and a DNA sequencer. Magnify this process many times over, with shed skin samples from a taxon all across its range, and you could put together quite an impressive phylogeographic study (that is, species range-wide population genetics studies applied to geography). But what about using the same technology to discover what individual reptiles do in their everyday lives, from season to season, and from one life history stage to another?

Scatology, the study of diet by examining feces, has received a makeover in its newest incarnation, *molecular scatology*. Recently, at least two studies have used this technology to identify the prey eaten by lizards and snakes in the wild (Brown *et al.* 2013; Brown, Ebenezer, and Symondson 2013). Animals are captured, and fecal samples can be taken *in vivo*, extracted of their DNA, and then applied to a "shotgun" approach to PCR with the aim of indentifying prey using a broad sweep of primers specific to candidate invertebrate, reptilian, mammalian, avian, amphibian, and fish sequences, among others. Snake ecologist Harry Greene (pers. comm.) of Cornell University has pointed out such methods do have their limitations. For instance, much data would not be attainable, like how many individual prey, prey size, and direction or orientation of ingestion, when compared to stomach content examination methods. Nevertheless, the prospect of identifying diet by examining fecal samples has its uses. It almost makes me daydream about coaxing a captured hatchling Texas Lyre Snake (*Trimorphodon biscutatus vilkinsonii*) to poop on me, just so I can understand something about the lives of these rarely encountered youngsters.

In Closing

This article started out with a hat tip to Carl Sagan. As a final nod in that direction, I wish to close "Part 2" by saying that these are but a few of the kinds of herpetological questions molecules can answer, given 4 billion years of evolution of those same molecules raised to consciousness in an obscure, unlikely vessel called *Homo sapiens*. We are indeed a way for the cosmos to know itself and our earthly kin.

And yet, in the grand cosmic scheme of things, I can't help but (quite vulnerably) feel our methods, as new as they are, are pitifully primitive and feeble. Even so, my greatest struggle as a student is to understand them. Simply being emotionally moored to the human baggage and inadequacies to which we are helplessly tethered—as strong as that force is—is not enough to dissuade us from using the blunt tools we have to keep probing, to keep being pioneers, pressing onward into darkness. The wild horses of our primordial heritage loom about—on one hand, endowing us with penetrating minds; and on the other, simultaneously threatening to drag us away from a potentially, unimaginably bright future. But I take comfort in the fact that others are quicker studies than myself, able to lap up abstract concepts and share them. Though mawkish, it is in that mutualistic spirit that I share. I look forward to future installments.

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Finally, I would be negligent if I didn't thank (and sincerely apologize to) Colton for his patience while I typed away, instead of playing with him. Children are humanity's 'default setting' for having priorities straight, and I'm grateful for his teaching me that.

The author assumes full responsibility for all statements and errors made herein.

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Update on the Distribution of the Rio Grande Chirping Frog, *Eleutherodactylus cystignathoides campi*, in the United States (Anura: Eleutherodactylidae)

by Tom Lott

Rio Grande Chirping Frog (*Eleutherodactylus cystignathoides*) from Beaumont, Jefferson County, Texas. Photo by Neil Fogal.

I have previously argued (Lott 2012) that more accurately depicting the rapidly expanding, dynamic distribution of the Rio Grande Chirping Frog (*Eleutherodactylus cystignathoides campi*) required using sources typically avoideded by more established methods but that were otherwise reasonable, including the provisional acceptance of records lacking officially recognized voucher specimens, photos, or recordings. This particularly includes such "citizen science" efforts as the Herpetological Education and Research Project (H.E.R.P.) database and the Herps of Texas (H.O.T.) project within the extensive iNaturalist.org online database. This is also an opportune time to summarize recent range extensions appearing in the peer-reviewed media and various books that might go otherwise unnoticed.

In this update I note fourteen counties in Texas (11 new counties, plus 3 upgraded from provisional status), 1 additional parish in Louisiana, and 1 county in the state of Alabama that have been added to the known distribution of this species in the US.

It has also come to my attention the term "invasive" has acquired a pejorative connotation, implying that any exotic species exerts a deleterious effect on the native fauna or environment it has "invaded." To my knowledge, such has not been shown to be the case with the Rio Grande Chirping Frog, even in areas where it now occurs in more-or-less natural (i.e., not human-disturbed) situations. Consequently, this species should be considered merely "exotic," rather than "invasive," until evidence is offered indicating that its presence has resulted in negative environmental consequences. Neither should any exotic species automatically be considered harmful until data are presented to support the assertion.