



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

# Clinical Aspects of Evolution in Reptile Medicine

James F.X. Wellehan, Jr.

## EVOLUTION

Evolution is an essential concept in biology. Indeed, when one considers definitions for life, perhaps the simplest and most elegant definition is that life consists of things that evolve. As medicine is a subfield of biology, evolution is central.

In comparative medicine, we often lack information on a given species, in areas including anatomy, physiology, microbiology, and pharmacology. When information is lacking on a given species, the best model to use is typically the closest relative from which data are available. This requires knowledge of species relationships. Because of ethical concerns, humans are not commonly used for experimentation; some common and appropriate laboratory models used are other primates or the closest relatives of the primates, rabbits and rodents. However, the understanding of animal classification that most current adults have been taught in grade school is fundamentally erroneous. Many commonly used terms, such as *reptile* or *lizard*, as they are generally interpreted, can lead to misunderstanding of relationships.

A definition is first necessary (Box 3-1). A *monophyletic group* is defined as a group that contains a common ancestor and all descendants. In comparative medicine, understanding what constitutes a monophyletic group is needed to understand relationships and to choose appropriate models. A group that is not monophyletic is called *paraphyletic*. Paraphyletic groups may not share a common ancestor or may exclude some descendants of the common ancestor. It is illogical to predict that paraphyletic groups would share characteristics that are not in nonmembers. Primates constitute a monophyletic group; when the primates except for humans are referred to, the term *nonhuman primates* is generally used. This qualification in the term helps the reader understand that a paraphyletic group is being referred to.

## EVOLUTION OF TETRAPODS

When the evolution of the tetrapods (terrestrial vertebrates) is examined, both the fossil record and the even stronger evidence from nucleic acid sequence phylogeny analyses are in agreement on relationships.<sup>1</sup> The earliest divergence among the tetrapods is between the amphibians and the amniotes (Figure 3-1). The amniotes consist of the reptiles (including birds) and the mammals. The amnion was a major evolutionary advance, enabling the amniotes to have a completely terrestrial life cycle without the need to return to water for

reproduction. This is also the first place in tetrapod evolution where common knowledge gives us fundamental errors. Herpetology is the study of nonavian reptiles and amphibians; it does not include mammals and birds. However, nonavian reptiles are far more fundamentally similar to birds and mammals than they are to amphibians, so it is not reasonable to expect nonavian reptile biology to resemble amphibian biology more closely than avian biology or mammalian biology. This is most medically apparent when looking at skin function, respiration, or reproduction.

## AMNIOTES

The amniotes are then further divided into mammals and sauropsids (see Figure 3-1, shown in green). Within the sauropsids, the first group to diverge is the squamate (lizards and snakes)/sphenodontid (tuatara) clade. After this, the testudines (turtles) diverged, and the last two major sauropsid groups to diverge were the crocodylians and the dinosaurs, collectively known as the archosaurs. The recognition of dinosaurs as reptiles is widespread in our culture. However, what is not generally recognized is that dinosaurs are not extinct and that birds are the only surviving group of dinosaurs. This is supported both by the fossil record and by sequence data.<sup>2</sup> Part of this failure may be due to an erroneous picture of nonavian dinosaurs: they shared a number of traits with birds, including feathers,<sup>3</sup> and there is evidence they were warm blooded.<sup>4</sup> Nevertheless, although birds themselves constitute a monophyletic group, if birds are not considered to be part of the reptile group, then reptiles are not a monophyletic group. What we really mean by reptiles is sauropsids, and birds are a group of reptiles. The term *nonavian reptile* should be used if excluding birds from the reptiles, an awkward term indicating a logically awkward paraphyletic group.

## CROCODYLIANS AND BIRDS

Crocodylians and birds share a number of medically relevant similarities. In a mammal, a persistent right aortic arch is a developmental problem that obstructs the esophagus, and the left aortic arch is the main outflow for oxygenated blood from the heart. In archosaurs, the right aortic arch is the major outflow.<sup>5</sup> Unlike birds, crocodylians have retained their left aortic arch, but the function it serves is primarily to transport hypercapnic blood to the stomach, where it is used to create

## BOX 3-1 DEFINITIONS

**Agamids:** The clade of lizards containing Bearded Dragons, Water Dragons, *Uromastix* sp., and related species. Agamids are members of the larger clade Toxicofera.

**Amniote:** The clade of animals who undergo embryonic development in an amnion, consisting of the sauropsids and the mammals. The evolution of the amnion enabled reproduction in arid environments.

**Amphisbaenids:** The clade of squamates that are often called Worm Lizards. Many are legless.

**Anguids:** The clade of lizards containing Glass Lizards, Alligator Lizards, and related species. Anguids are members of the larger clade Toxicofera.

**Archosaurs:** The clade of reptiles whose surviving members are the crocodiles and birds.

**Clade:** A monophyletic group.

**Definitive host:** A host in which a pathogen has adapted to successfully propagate.

**Dinosauria:** The clade of reptiles containing the birds. Nonavian dinosaurs went extinct by the end of the Cretaceous period, 65 million years ago.

**Helodermatids:** The clade of lizards containing Gila Monsters and Beaded Lizards. Helodermatids are members of the larger clade Toxicofera.

**Homeothermy:** The trait of maintaining a constant body temperature, often above that of the environment.

**Lacertids:** The clade of lizards containing Jeweled Lacertas, Wall Lizards, and related species.

**Monophyletic:** Adjective describing a group that contains a common ancestor and all the descendants. A monophyletic group is also known as a clade.

**Mutualistic:** An ecologic relationship that benefits both organisms involved.

**Paraphyletic:** Adjective describing a group that is not monophyletic; the group's members do not share a common ancestor or exclude some descendants of a common ancestor.

**Poikilothermy:** The trait of depending on the environment for temperature regulation.

**Sauropsid:** The clade consisting of the reptiles, including squamates, tuataras, testudines, dinosaurs, and crocodylians. The term *reptiles* is often misunderstood not to include modern dinosaurs.

**Sphenodontid:** The reptile clade whose last surviving members are the tuatara in New Zealand.

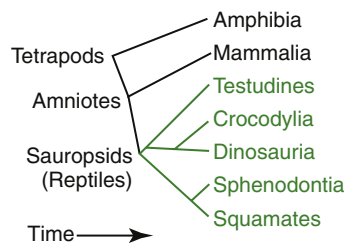
**Squamate:** The clade of reptiles consisting of the lizards (including snakes).

**Teiids:** The clade of lizards containing tegus, ameivas, whiptails, caiman lizards, and related species.

**Testudines:** The clade of reptiles consisting of the turtles.

**Tetrapod:** The clade of animals containing all terrestrial vertebrates, including the reptiles, amphibians, and mammals. Along with lungfish and coelacanth, tetrapods are members of the larger clade Sarcopterygii (lobe-finned fish).

**Toxicofera:** The clade of lizards that possess venom apparatus, including snakes, iguanids, agamids, chameleons, monitors, helodermatids, and anguids.



**FIGURE 3-1** Phylogenetic tree of the tetrapods. Reptiles are in green.

an extraordinarily low gastric pH.<sup>6</sup> Indeed, crocodylians are one of the few groups of animals studied in which oral fluoroquinolones do not result in good blood levels, and it is possible that breakdown in the extremely acidic stomach may play a role in this.<sup>7,8</sup>

Both birds and crocodylians have four-chambered hearts. A four-chambered heart is not needed for separation of right and left blood flow, which is accomplished in nonarchosaur reptiles that lack a ventricular septum.<sup>9</sup> The major advantage of a ventricular septum is facilitation of pressure differentials between the left and right sides of the heart, as may be needed in an animal with a high metabolic rate. Ventricular septa evolved separately in archosaurs and mammals; both groups have warm-blooded members.

Crocodylians and birds also both have in common a respiratory system with unidirectional rather than tidal air flow, a much more efficient design than the mammalian lung.<sup>10</sup> An

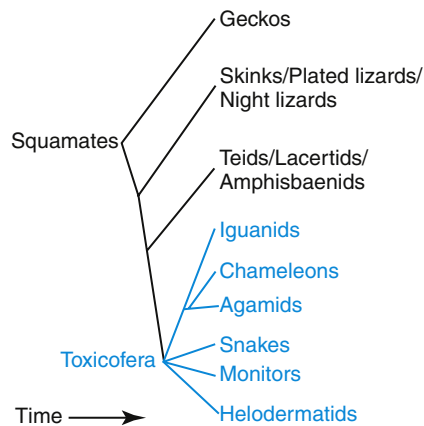
efficient respiratory system is also needed for a higher metabolic rate.

Ancestors of modern crocodylians from shortly after the divergence from dinosaurs were morphologically consistent with long-legged active terrestrial predators.<sup>11</sup> It is plausible that ancestral archosaurs were warm-blooded animals and that modern crocodylians lost homeothermy, which would be an evolutionarily disadvantageous trait for aquatic ambush predators.

When examined in detail, significant homologies between crocodylians and their closest extant relatives, the birds, become clear. However, the significant differences between birds and crocodylians are also obvious, and the clinician needs to be careful not to overextrapolate. The obvious differences between an alligator and a Fulvous Whistling Duck underscore the more cryptic but greater differences between an alligator and a tegu.

## LIZARDS

Another prevalent error is the concept of lizards as a group distinct from snakes. In squamate evolution, the earliest divergence is the geckos, followed by the divergence of the skinks, night lizards, and plated lizards (Figure 3-2). The next groups to branch off were the teiids, lacertids, and amphisbaenids, and the remaining group, containing snakes, iguanids, agamids, chameleons, monitors, helodermatids, and anguids, is known collectively as the Toxicofera (see blue on Figure 3-2), named for the commonality of the presence of venom glands. There is



**FIGURE 3-2** Phylogenetic tree of the squamates. Toxicofera are in blue.

a very nice image of the histologic features of Bearded Dragon venom glands in Fry et al.<sup>12</sup> Snakes diverge in the middle of the squamates, and if snakes are removed, then lizards are not a monophyletic group. Snakes are a group of lizards, and a Cornsnake is a better model for a Bearded Dragon than a Leopard Gecko.

## CARING FOR CAPTIVE ANIMALS

It is critical, when caring for captive animals, to consider environments in which they have evolved. Eons of selective pressure have resulted in reptiles that have adapted to specific diets, habitats, and threats, and disease may result when captive conditions differ. Periodontal disease is common in lizards with acrodont dentition (agamids and chameleons) in captivity but not in the wild.<sup>13</sup> Infiltrative lipomas appear to be common in obese captive Cornsnakes.<sup>14</sup> Significant rostral trauma is common in water dragons and basilisks kept in glass enclosures without sufficient cover.

## INFECTIOUS DISEASES

Evolution is also central to infectious disease. Multiple factors influence evolutionary rates, including selective pressures, generation times, and fidelity of copying genes. Microbes often have very short generation times. Ribonucleic acid (RNA) viruses, generally lacking proofreading, have high error rates when they make copies. As a result, evolution rates in microbes tend to be rapid, and RNA viruses are the most rapidly evolving organisms on the planet. This is useful for rapid adaptation to novel selective pressures, such as immune selection and antimicrobial use. To compensate, the most rapidly evolving genes in vertebrates are immune related.

## MICROBES

Microbes are essential for all vertebrate life, for functions including digestion, nutrition, and defense. In veterinary school, we have all been taught Koch's postulates as criteria for establishing a microbe as a pathogen. Although Koch's postulates have their use, they frequently result in a false dichotomous understanding of microbes as either pathogenic or nonpathogenic. There is no such thing as a microbe that is

always either a pathogen or a nonpathogen. There have been many asymptomatic human Ebola virus infections, and people have died of septicemia due to *Lactobacillus acidophilus*.<sup>15,16</sup> A microbe does not "want" to cause disease or not cause disease. All life on earth has been selected for billions of years to reproduce successfully, and this is all that matters from an evolutionary standpoint. If pathogenic traits provide an evolutionary advantage in a given situation, they will be selected for. If they provide a disadvantage, they will be selected against.

A number of important selective pressures affect microbes in a vertebrate host, including nutrient availability, temperature, competition with other microbes, the need to transfer to a new host, and the host immune system. A vertebrate host is a nutrient-rich environment. However, some nutrients may be sequestered; significant resources are spent by the host synthesizing transferrin, lactoferrin, and ferritin to make it unavailable. Many bacterial virulence pathways have evolved to access this sequestered iron.<sup>17,18</sup>

Homeothermic vertebrates also provide a highly temperature-controlled environment, whereas poikilothermic hosts require the ability to survive at different temperatures. Because of this, infectious disease manifestation may be highly temperature-dependent in poikilotherms.<sup>19-21</sup> This adds an extra dimension to disease ecology, especially with latent or persistent infections. Further investigation of the role of temperature in disease manifestation in poikilotherms is strongly indicated, especially with populations of many reptile species critically declining and likely to be affected by anthropogenic climate change.<sup>22</sup>

Competition is also a major selective pressure in a vertebrate; many organisms want to live in such a nutrient-rich environment. The majority of antimicrobials are derived from molecules secreted by other microbes to compete for ecologic niches. Animal guts are some of the most diverse and rich ecosystems to be found anywhere. Many organisms that have evolved in such a competitive environment have resistance to many antimicrobials; *Enterococcus* sp. is a classic example.

The limited lifespan of vertebrate hosts creates significant selective pressure toward the ability to move on to a new host. This often involves secretion of large amounts of microbes via respiratory discharge or diarrhea, but other routes occur, such as the simultaneous behavioral changes and salivary gland shedding of rabies or the use of insect vectors. Three fundamental strategies can be used to deal with limited host life spans. First, a microbe may survive well in the environment. Second, a microbe may adapt to a balance with the host environment. Finally, a microbe may move quickly to a new host.

Parasites often adapt to a balance with their hosts. Many parasites tend to have slower generation times compared with viruses or bacteria, which makes rapid reproduction and moving on to a new host less of a viable strategy. The costs of parasites to their definitive hosts are relatively minimal in many cases because it is advantageous to parasites to preserve their habitat. Bullfrog tadpoles carrying the pinworm *Gyrodactylus* have better feed conversion and metamorphose earlier than uninfested controls, rendering the relationship mutualistic rather than parasitic.<sup>23</sup> However, for parasites with indirect life cycles, causing disease in an intermediate host may be advantageous. If a rodent carrying pentastomid larvae is debilitated, it is more likely to be eaten by a snake, completing the life cycle. This may also result in greater disease

in accidental hosts.<sup>24</sup> Some parasites do survive well in the environment; this reduces the selective pressure not to harm the host. Parasites that survive well in the environment are much more likely to cause significant disease; perhaps the best known examples of this in reptiles are *Rhabdias* sp., which cause pulmonary disease in squamates that may be fatal.<sup>25</sup>

Most fungi also survive well in the environment, resulting in little selective pressure to keep their host alive. They compete significantly with bacteria for the same niches; this has resulted in the production of antibacterial compounds by fungi and antifungal agents by bacteria. The fungi are some of the closest relatives of animals; fungi, choanoflagellates, and metazoa (multicelled animals) form a clade known as the Opisthokonta.<sup>26</sup> An animal is much more closely related to a mushroom than it is to an oak tree. Antimicrobial drugs generally exploit differences in chemistry and metabolism between pathogen and host. Because fungi and vertebrate hosts diverged more recently, there are fewer differences to exploit, and antifungal drugs tend to have narrower therapeutic indices and use a smaller subset of mechanisms.

Bacteria constitute a large portion of the host ecosystem. There are far more bacterial cells in a normal vertebrate than there are vertebrate cells. Traditional approaches to examining bacterial diversity have depended on culture; this is a poor way of assaying diversity. Culture-independent methods, such as 16S polymerase chain reaction (PCR) and cloning, or high-throughput sequencing methods, have revealed that standard culture-based methods will detect between 1% and 10% of bacterial species present in most ecologic niches. As an understanding of further diversity has arisen, it becomes clearer that a vertebrate is a complex ecosystem.<sup>27</sup> This system may be very dynamic. The gut flora of Burmese Pythons changes significantly in response to feeding.<sup>28</sup> Postprandially, bacteria in the phylum Firmicutes (“classic” gram-positive bacteria, containing organisms such as *Clostridium*, *Lactobacillus*, and *Peptostreptococcus*) increase dramatically, while those in the phylum Bacteroidetes (primarily containing anaerobic gram-negative organisms such as *Bacteroides* and *Prevotella*) make up a greater percentage of the fewer species present after fasting. The gut flora did not significantly share species with prey mice, with the exception of the temporary postprandial establishment of *Lactobacillus* sp.

Ecologic disturbance may have significant negative impacts on many aspects of health. Damage to healthy gut flora by antibiotic use provides opportunity for invasive species; recent treatment with antibiotics markedly increases host susceptibility to *Salmonella*.<sup>29</sup> A 5-day course of ciprofloxacin will change human gut flora diversity and composition for several weeks, and the original composition may never reestablish.<sup>30</sup> In many ways, the use of broad-spectrum antibiotics for a bacterial infection in a vertebrate is analogous to starting a forest fire to get rid of coyotes. The ideal treatment for a bacterial pathogen would be as narrow-spectrum as possible, minimally disturbing the rest of the host ecosystem. Isoniazid, which targets only *Mycobacterium tuberculosis* and a few very closely related species and does not significantly affect many other *Mycobacterium* sp., is an excellent example. Unfortunately, current market forces have resulted in pharmaceutical companies developing antibiotics with as broad a spectrum as possible, and narrow spectrum antibiotics are often not put through further development and clinical trials.

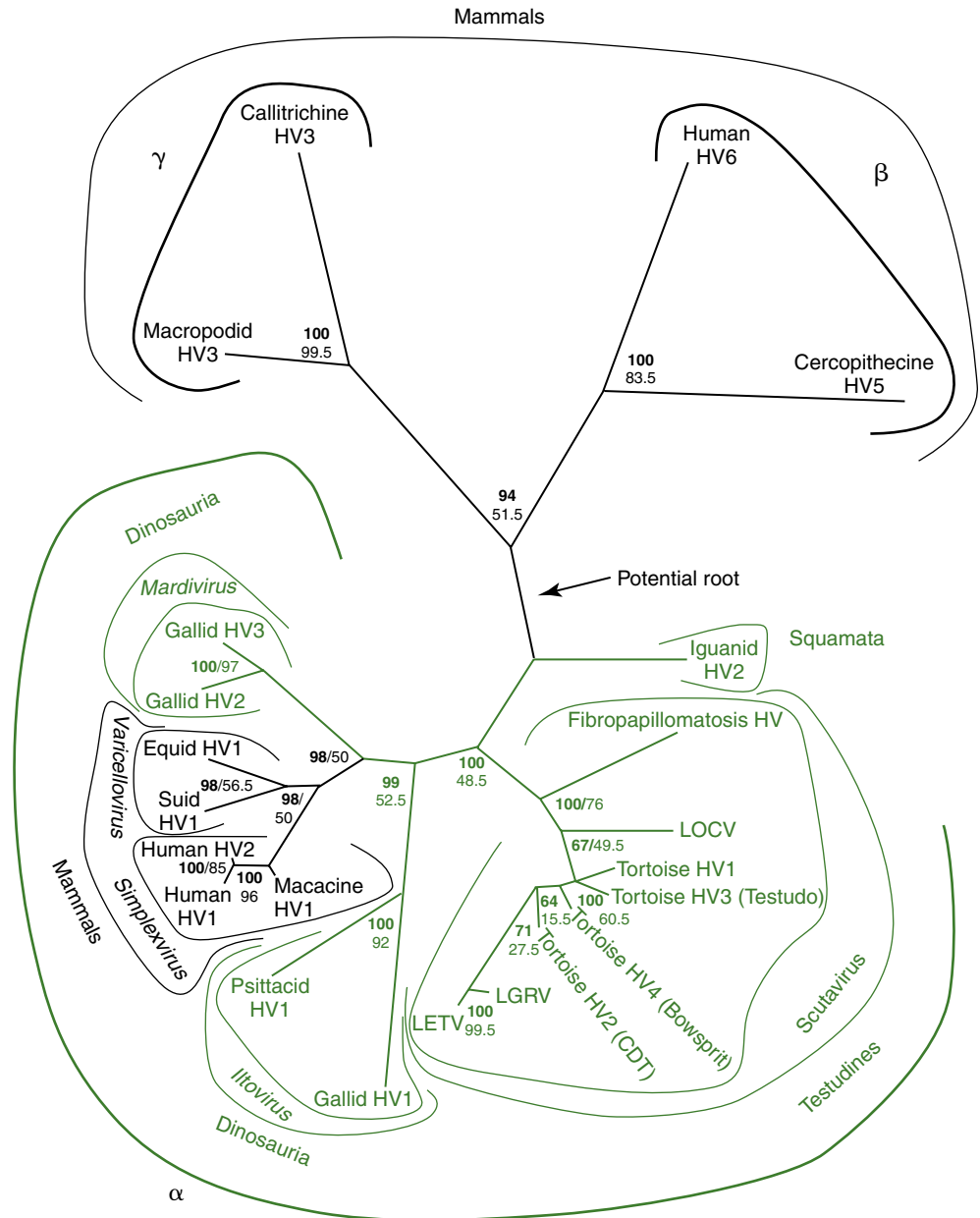
Antibiotic use without consideration of microbial ecology and evolution rapidly leads to failure. Back in the 1980s, gentamicin was promoted for eradication of *Salmonella* from turtles.<sup>31</sup> Over the next few years, the *Salmonella* isolates from farmed turtles acquired a high rate of gentamicin resistance<sup>32</sup> and therefore posed a greater risk to human health than they had previously. Several studies have suggested that wild turtles may have a lower carriage rate for *Salmonella*.<sup>33,34</sup> The only realistic way to reduce the risk of *Salmonella* in farmed turtles over the long term is to alter the ecologic niche that it inhabits. Keeping farmed animals in high population densities increases contact rates, pathogen loads, and stress, lowering barriers to transmission. Increased ease of transmission reduces the selective pressure to keep the host alive and healthy. Reducing the risk of *Salmonella* in hatchling turtles probably means farming at lower densities and eliminating high-density turtle farming.<sup>35</sup>

## VIRUSES

Viruses are strictly dependent on host cells for replication; this means that living in the environment as a strategy for dealing with limited host life spans is not a viable option. There are a number of important properties that affect viral evolution and ecology. Enveloped viruses are surrounded by a lipid envelope. This envelope is usually essential for invading a host cell. It is also easily damaged, which makes disinfection easier when dealing with an enveloped virus. Segmentation of viral genomes, allowing reassortment, may provide a hybrid advantage for crossing host species; this has been best studied in the Orthomyxoviridae.<sup>36</sup> Acquiring genes that are functional from a related virus is significantly more likely to be advantageous than random mutations; this is why animals and plants have sex. Throughout biology, hybridization is a factor allowing rapid nondetrimental change, allowing species to invade novel habitats.<sup>37</sup> New sites of infection or host species are novel virus habitats.

Nucleic acid type is another property with a major impact on viral evolution and ecology. Large deoxyribonucleic acid (DNA) viruses often adapt to a balance with their hosts. This usually involves latency or chronic infection, with a delicate balance with the host immune system. A larger number of genes is often used to maintain this balance. Because the viruses are larger and more complicated, they require more accurate replication to avoid accumulating lethal mutations. DNA viruses usually have much more accurate replication, with either host or viral proofreading mechanisms in place. Many DNA viruses evolve at rates not much more rapid than their hosts, which enables larger viral genomes with greater numbers of genes. Large DNA viruses, especially those with intranuclear replication, are the most host-specific viruses.<sup>38</sup> There is evidence that some of these viruses have codiverged evolutionarily along with their hosts; this evidence is strongest in the adenoviruses and herpesviruses. In Figure 3-3, a herpesvirus phylogenetic tree is shown. A potential root can be placed where the arrow is marked, representing an ancestral herpesvirus in an ancestral amniote. The earliest amniote divergence is between mammals and reptiles, as seen in Figure 3-1. All known members of the Betaherpesvirinae and Gammaherpesvirinae use mammal hosts, and the longer branch lengths in this area indicate that these viruses have diverged over a

**FIGURE 3-3** Phylogenetic tree of the Herpesviridae. Herpesviruses of reptile hosts are in green. Note similarity of branching pattern to that of the hosts.



longer period of time. Heading toward the Alphaherpesvirinae, the first agents to diverge infect squamates; the squamates are the earliest divergence within the reptiles. The next group to diverge is the scutavirus, infecting turtle/tortoise hosts; this is also consistent with host divergence patterns. There are no reliable crocodylian herpesvirus sequences available for comparison. *Mardivirus* and *Iltovirus* infect avian hosts. However, the mammalian alphaherpesviruses nest within the clade infecting avian hosts. The branch lengths within the mammalian alphaherpesviruses are relatively short, which indicates that these viruses have not diverged from each other to the same extent that mammalian herpesviruses in the other subfamilies have. One plausible explanation for this is that the mammalian alphaherpesviruses represent a host jump to mammals from the Dinosauria. Chickenpox, caused by the alphaherpesvirus human herpesvirus 3, may be a descendant of a dinosaurian virus and may be more aptly named than had been realized.

The complex coadaptation of some large DNA viruses provides selective advantage to causing minimal pathology in their hosts. A long-lived host may provide a suitable habitat for decades. However, this balance in a definitive host may not apply to other hosts. Hosts that are similar enough for a virus to infect but dissimilar enough for the intricate balance of latency/chronicity not to work may result in overwhelming and often fatal infection. The most significant pathology associated with herpesviruses is in aberrant hosts. A well-balanced host–virus relationship may actually be beneficial to the host. Columboid herpesvirus 1, endemic in Rock Doves, causes disease in squabs kept in stressful conditions, but the overall pathologic damage is relatively minimal. However, in raptors, which prey on Rock Doves, Columboid herpesvirus 1 causes an overwhelming infection that is rapidly fatal.<sup>39</sup> The advantage to the pigeon populations of killing off predators likely outweighs the disadvantage of minor disease in neonates.

RNA viruses reproduce less accurately. They generally lack proofreading and have the highest mutation rates of any organisms on the planet. These mutation rates mean that genetic complexity is not possible; the high error rates would render progeny requiring a large gene set nonviable. RNA viruses have small genomes and fewer genes. The advantage of such a high error rate is that RNA viruses are capable of rapidly outmaneuvering the host immune system. The strategy of RNA viruses is more likely to be rapid reproduction and moving to a new host. Because they have less complex relationships with their hosts, RNA viruses are much more capable of moving to new host species. This ability to move to new hosts reduces the selective pressure not to harm the host, and many RNA viruses are more pathogenic. A metaanalysis found that of the 20 virus families infecting the best-studied vertebrate host species, humans, four RNA virus families, Reoviridae, Bunyaviridae, Flaviviridae, and Togaviridae, accounted for more than half of emerging and reemerging viruses.<sup>40</sup> When one considers major viral human diseases that have recently emerged, high-profile diseases such as severe acute respiratory syndrome (SARS) (Coronaviridae), West Nile (Flaviviridae), influenza (Orthomyxoviridae), Ebola (Filoviridae), and Hendra (Paramyxoviridae) are all RNA viruses.

The retroviruses have RNA genomes and, when actively replicating, have very high mutation rates similar to other RNA viruses. However, retroviruses are unusual in that they reverse transcribe from RNA to DNA, and the DNA copy of their genome is then incorporated into the host genome. This has happened a lot over the course of evolution and makes retroviral discovery and diagnosis very challenging, not because they are hard to find but because they are widespread and present in such large numbers that it is difficult to sort out disease-associated virus from clinically irrelevant endogenous virus. Approximately 1% of the typical vertebrate genome encodes for vertebrate proteins, whereas 8% to 9% of the vertebrate genome is retroviral in origin. The diversity of retroviruses in reptiles is only beginning to be understood.<sup>41</sup> Because of the prevalence of retroviruses in their genomes, reverse transcriptase, the enzyme that converts viral RNA back to DNA, is commonly expressed in host cells. This has also resulted in less frequent incorporation of other viruses into host genomes, especially those that replicate in the nucleus. Bornaviruses, which have the uncommon trait for RNA viruses of nuclear replication, have been found to be incorporated into the genomes of many vertebrates, and bornaviral cDNA has been found in Gaboon Vipers.<sup>42</sup> Incorporation of inactive virus into host genomes complicates interpretation of nucleic acid–based diagnostics.

### PATHOGENIC DISEASES

Several routine husbandry practices in the reptile trade create strong evolutionary selective pressures toward pathogenicity. First, overcrowding is common. It is unfortunately common and considered acceptable to house snakes in breeder racks, where enclosures that are smaller in dimension than the length of the snake are stored in vertical racks in close proximity. The stress of close confinement results in elevated corticosteroids and immunosuppression.<sup>43</sup> The design of these breeder racks makes good biosecurity practices impossible. High population densities lower transmission barriers, reducing pressure to keep hosts alive and selecting toward virulence.<sup>44</sup> It is also

common in the reptile trade to select for color phases. This usually involves some degree of inbreeding to select for what are often recessive traits. A major driving force for the evolution of sex is acquisition of genetic diversity for immune function. Inbreeding results in selection for greater disease.<sup>45</sup> Finally, reptiles are still often wild-caught, and stressed wild-caught animals are brought to large distributors who have species from all over the world in the same facility with insufficient biosecurity. This is an ideal situation for pathogen host jumps, which is where the most dramatic disease is seen.<sup>46</sup> Mixing of species by the exotic animal trade has already proved disastrous, with the transferral of monkeypox from Gambian Pouched Rats to Prairie Dogs to humans.<sup>47</sup> An orthoreovirus was first isolated in 1996 from a Mediterranean Spur-thighed Tortoise (*Testudo graeca*) in Switzerland and later characterized by sequencing.<sup>48</sup> This virus has more recently been associated with a high mortality rate and syncytial cell enteropathy/hepatopathy in Leopard Geckos widely distributed in the United States.<sup>49</sup> This almost certainly represents a host jump at a breeding institution or distributor.

Reduction of the significant selective pressures toward highly pathogenic diseases involves major changes in the reptile industry. Genetic diversity in populations needs to be valued and monitored through appropriate use of studbooks and cooperative rather than competitive interactions with breeders. Breeding for mutations needs to be discouraged. Housing needs to be entirely revised so that larger enclosures for individual animals allow feeding and cleaning to be done without cross-contamination to other animals. Importation of wild animals for sale as pets needs to be strongly discouraged. Facilities need to focus on single species and have smaller numbers of animals at lower densities.

In conclusion, evolution is central to all areas of comparative medicine. It is critical for the reptile practitioner to take this into account, especially when dealing with herd health and infectious diseases.

### REFERENCES

- Hugall AF, Foster R, Lee MS. Calibration choice, rate smoothing, and the pattern of tetrapod diversification according to the long nuclear gene RAG-1. *Syst Biol* 2007;56:543–563.
- Schweitzer MH, Zheng W, Organ CL, et al. Biomolecular characterization and protein sequences of the Campanian hadrosaur *B. canadensis*. *Science* 2009;324:626–631.
- Xu X, Wang K, Zhang K, et al. A gigantic feathered dinosaur from the lower cretaceous of China. *Nature* 2012;484:92–95.
- Eagle RA, Tütken T, Martin TS, et al. Dinosaur body temperatures determined from isotopic (<sup>13</sup>C-<sup>18</sup>O) ordering in fossil biominerals. *Science* 2011;333:443–445.
- Eme J, Gwalhney J, Owerkowicz T, et al. Turning crocodylian hearts into bird hearts: growth rates are similar for alligators with and without right-to-left cardiac shunt. *J Exp Biol* 2010;213:2673–2680.
- Farmer CG, Uriona TJ, Olsen DB, et al. The right-to-left shunt of crocodylians serves digestion. *Physiol Biochem Zool* 2008;81:125–137.
- Helmick KE, Papich MG, Vliet KA, et al. Pharmacokinetics of enrofloxacin after single-dose oral and intravenous administration in the American alligator (*Alligator mississippiensis*). *J Zoo Wildl Med* 2004;35:333–340.
- Martelli P, Lai OR, Krishnasamy K, et al. Pharmacokinetic behavior of enrofloxacin in estuarine crocodile (*Crocodylus porosus*) after single intravenous, intramuscular, and oral doses. *J Zoo Wildl Med* 2009;40:696–704.

9. Jensen B, Nielsen JM, Axelsson M, et al. How the python heart separates pulmonary and systemic blood pressures and blood flows. *J Exp Biol* 2010;213:1611–1617.
10. Farmer CG, Sanders K. Unidirectional airflow in the lungs of alligators. *Science* 2010;327:338–340.
11. Riff D, Kellner AWA. Baurusuchid crocodyliforms as theropod mimics: clues from the skull and appendicular morphology of *Stratiotosuchus maxhechti* (Upper Cretaceous of Brazil). *Zool J Linnean Soc* 2011;163:S37–S56.
12. Fry BG, Vidal N, Norman JA, et al. Early evolution of the venom system in lizards and snakes. *Nature* 2006;439:584–588.
13. McCracken HE. Periodontal Disease in Lizards. In: Fowler ME, Miller RE, Donley S, eds. *Zoo and wild animal medicine: current therapy*. 4th ed. Philadelphia: WB Saunders Co, 1998;252–257.
14. Burkert, BA, Tully TN, Nevarez J, et al. Infiltrative lipoma in a corn snake, *Elaphe guttata guttata*. *J Herpetol Med Surg* 2002;12(3):33–35.
15. Leroy EM, Baize S, Volchkov VE, et al. Human asymptomatic Ebola infection and strong inflammatory response. *Lancet* 2000;355:2210–2215.
16. Cannon JP, Lee TA, Bolanos JT, et al. Pathogenic relevance of *Lactobacillus*: a retrospective review of over 200 cases. *Eur J Clin Microbiol Infect Dis* 2005;24:31–40.
17. Haley KP, Skaar EP. A battle for iron: host sequestration and *Staphylococcus aureus* acquisition. *Microbes Infect* 2012;14:217–227.
18. Perry RD, Fetherston JD. Yersiniabactin iron uptake: mechanisms and role in *Yersinia pestis* pathogenesis. *Microbes Infect* 2011;13:808–817.
19. Klenk K, Snow J, Morgan K, et al. Alligators as West Nile virus amplifiers. *Emerg Infect Dis* 2004;10:2150–2155.
20. Rojas S, Richards K, Jancovich JK, et al. Influence of temperature on Rana virus infection in larval salamanders *Ambystoma tigrinum*. *Dis Aquat Organ* 2005;63:95–100.
21. Goodwin AE, Merry GE. Mortality and carrier status of bluegills exposed to viral hemorrhagic septicemia virus genotype IVb at different temperatures. *J Aquat Anim Health* 2011;23:85–91.
22. Slenning BD. Global climate change and implications for disease emergence. *Vet Pathol* 2010;47:28–33.
23. Pryor GS, Bjornndal KA. Effects of the nematode *Gyrodactylus* on development, gut morphology, and fermentation in bullfrog tadpoles (*Rana catesbeiana*): a novel mutualism. *J Exp Zool A Comp Exp Biol* 2005;303:704–712.
24. Brookins MD, Wellehan JF, Roberts JF, et al. Massive visceral pentastomiasis caused by *Porocephalus crotali* in a dog. *Vet Pathol* 2009;46:460–463.
25. Langford GJ, Janovy J Jr. Comparative life cycles and life histories of North American *Rhabdias* spp. (Nematoda: Rhabdiasidae): lungworms from snakes and anurans. *J Parasitol* 2009;95:1145–1155.
26. Torruella G, Derelle R, Paps J, et al. Phylogenetic relationships within the Opisthokonta based on phylogenomic analyses of conserved single copy protein domains. *Mol Biol Evol* 2012;29:531–544.
27. Robinson CJ, Bohannon BJ, Young VB. From structure to function: the ecology of host-associated microbial communities. *Microbiol Mol Biol Rev* 2010;74:453–476.
28. Costello EK, Gordon JI, Secor SM, et al. Postprandial remodeling of the gut microbiota in Burmese pythons. *ISME J* 2010;4:1375–1385.
29. Croswell A, Amir E, Teggatz P, et al. Prolonged impact of antibiotics on intestinal microbial ecology and susceptibility to enteric *Salmonella* infection. *Infect Immun* 2009;77:2741–2753.
30. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A* 2011;108:4554–4561.
31. Siebeling RJ, Caruso D, Neuman S. Eradication of *Salmonella* and *Arizona* species from turtle hatchlings produced from eggs treated on commercial turtle farms. *Appl Environ Microbiol* 1984;47:658–662.
32. Diaz MA, Cooper RK, Cloeckaert A, et al. Plasmid-mediated high-level gentamicin resistance among enteric bacteria isolated from pet turtles in Louisiana. *Appl Environ Microbiol* 2006;72:306–312.
33. Richards JM, Brown JD, Kelly TR, et al. Absence of detectable *Salmonella* cloacal shedding in free-living reptiles on admission to the wildlife center of Virginia. *J Zoo Wildl Med* 2004;35:562–563.
34. Saelinger CA, Lewbart GA, Christian LS, et al. Prevalence of *Salmonella* spp in cloacal, fecal, and gastrointestinal mucosal samples from wild North American turtles. *J Am Vet Med Assoc* 2006;229:266–268.
35. Izadjoo MJ, Pantoja CO, Siebeling RJ. Acquisition of *Salmonella* flora by turtle hatchlings on commercial turtle farms. *Can J Microbiol* 1987;33:718–724.
36. Macken CA, Webby RJ, Bruno WJ. Genotype turnover by reassortment of replication complex genes from avian influenza A virus. *J Gen Virol* 2006;87:2803–2815.
37. Rieseberg LH, Kim SC, Randell RA, et al. Hybridization and the colonization of novel habitats by annual sunflowers. *Genetica* 2007;129:149–165.
38. Pulliam JR, Dushoff J. Ability to replicate in the cytoplasm predicts zoonotic transmission of livestock viruses. *J Infect Dis* 2009;199:565–568.
39. Pinkerton ME, Wellehan JF Jr, Johnson AJ, et al. Columbiv herpesvirus-1 in two Cooper's hawks (*Accipiter cooperii*) with fatal inclusion body disease. *J Wildl Dis* 2008;44:622–628.
40. Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. *Emerg Infect Dis* 2005;11:1842–1847.
41. Jaratlerdsiri W, Rodríguez-Zárate CJ, Isberg SR, et al. Distribution of endogenous retroviruses in crocodylians. *J Virol* 2009;83:10305–10308.
42. Horie M, Honda T, Suzuki Y, et al. Endogenous non-retroviral RNA virus elements in mammalian genomes. *Nature* 2010;463:84–87.
43. Sykes KL, Klukowski M. Effects of acute temperature change, confinement and housing on plasma corticosterone in water snakes, *Nerodia sipedon* (Colubridae: Natricinae). *J Exp Zool A Ecol Genet Physiol* 2009;311:172–181.
44. Borovkov K, Day R, Rice T. High host density favors greater virulence: a model of parasite-host dynamics based on multi-type branching processes. *J Math Biol* 2012. doi: 10.1007/s00285-012-0526-9 [Epub ahead of print].
45. Morran LT, Schmidt OG, Gelarden IA, et al. Running with the Red Queen: host-parasite coevolution selects for biparental sex. *Science* 2011;333:216–218.
46. Pulliam JR. Viral host jumps: moving toward a predictive framework. *Ecohealth* 2008;5:80–91.
47. Bernard SM, Anderson SA. Qualitative assessment of risk for monkey-pox associated with domestic trade in certain animal species, United States. *Emerg Infect Dis* 2006;12:1827–1833.
48. Wellehan JF Jr, Childress AL, Marschang RE, et al. Consensus nested PCR amplification and sequencing of diverse reptilian, avian, and mammalian orthoreoviruses. *Vet Microbiol* 2009;133:34–42.
49. Garner MM, Farina LL, Wellehan JFX, et al. Reovirus-associated syncytial cell enteropathy and hepatopathy in leopard geckos, *Eublepharis macularius*. In *Proceedings*. Conference of the Association of Reptilian and Amphibian Veterinarians, Milwaukee, Wis, 2009;82.