Krogh's Principle and Why the Modern Zoo Is Important to Academic Research

Chapter · February 2019

DOI: 10.1017/9781108183147.023

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> For a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied.
>
> – The Krogh Principle, A. Krogh (1929)

August Krogh's (1929) classic essay invoked animal diversity as a panacea for modern research wants and needs. Therein, he sagely predicted a future of go-to model organisms for research and, in that context, clearly implicated the importance of considering outlying, bizarre creatures (reviewed by Lindstedt, 2014). Krogh was clairvoyant in his embrace of biological and disciplinary diversity to study animals, systems, and nature in order to produce the most robust conclusions – indeed, conclusions derived from multiple perspectives of not only the diversity of study organism(s), but also the diversity of the scientists considering them. He coined the phrase "comparative physiology" decades before the emergence of the comparative method (e.g., Harvey & Pagel, 1991) was formulated, and before academics even considered interdisciplinary research as somewhat novel (Collins, 2002; Pough, 1993). Despite its antiquated word choice, we take especial note of Krogh's (1929, p. 5) statement: "You will find in lower animals mechanisms and adaptations of exquisite beauty and the most surprising character [. . .]"

From this foundation, we will make the case that amphibians and reptiles, aside from the handful of included model organisms (e.g., *Xenopus* frogs or red-eared slider turtles), provide the menu of biological diversity that Krogh envisioned. The requisite taxonomic diversity – the veritable menu – for scientists of all disciplines to consider is most available and tractable in the form of amphibians and reptiles in zoos. Hence, we paraphrase Krogh (1929) in suggesting that zoos will become a necessary part of academic research in the future. We will support this thesis with examples from the published literature and our own experiences in zoos and academia.

ZOOS AND UNIVERSITIES: COMPLEMENTARY CONSTRAINTS AND OPPORTUNITIES

Zoos provide opportunities to observe "natural" (species-specific) behaviors that may be difficult or impossible to document in the wild. In 1960, George and Mary Rabb at Brookfield Zoo described the unusual mating behaviors of the Suriname toad (*Pipa pipa*). This species lives in shallow, muddy ponds in Amazonia and it is unlikely that these behaviors would ever have been fully observed in the wild. Nearly 60 years later, Robert Hill at Zoo Atlanta described herbivory in another swamp-dwelling amphibian, the greater siren (*Siren lacertina*), clarifying misconstrued reports from individuals collected as museum specimens and confirming this as the first species of salamander known to be omnivorous (Hill, Mendelson, & Stabile, 2015). Likewise, many academic researchers have taken advantage of the taxonomically broad collections at zoos in their basic research. For example, various aspects of snake feeding behaviors have been documented across a wide literature (e.g., Deufel & Cundall, 2003; Greene, 1976; Greene & Burghardt, 1978; Radcliffe, Chiszar, & O'Connell, 1980). While the habits of Komodo dragons (*Varanus komodoensis*) were well studied in the wild (Auffenberg, 1981), a great deal of their biology has also been discovered among zoo populations (reviewed in Murphy, Ciofi, de La Panouse, & Walsh, 2002; Watts et al., 2006). Unsurprisingly, the majority of the literature on herpetological veterinary medicine and reproduction has been conducted using zoo collections, as well as applied topics such as nutrition and general husbandry (see Murphy, Adler, & Collins, 1994). Nevertheless, 20 years ago, Card, Roberts, and Odum (1998) decried the unfortunate missed opportunities for herpetological research in zoos in that their own staff are either not encouraged, supported, or motivated to conduct formal research. Our view is that this problem remains largely unchanged to this day, and this is the impetus for this chapter.

Zoos maintain diverse collections for the sake of illustrating that very diversity. Universities, on the other hand, increasingly require that each individual and species maintained on campus must be demonstrably part of an active research project, and that such projects have timelines and exit strategies with regard to the final disposition of the animals. The opportunities to maintain a small, living collection on campus for teaching, or as a living reference collection for research, are largely gone. Furthermore, many universities will not allow venomous species to be maintained on campus for any purpose. Finally, many universities now require that live animals be maintained in a central, designated animal facility and cared for by dedicated staff. In our experience, university animal care staff are accustomed to caring for a short list of familiar model organisms, such as rats or *Xenopus*, and simply are not experienced in caring for other types of animals commonly labeled as exotic. We have seen this organizational model lead to problems in maintaining healthy reptiles and amphibians for research projects. Considered together, these policies place rather severe limits on the abilities of university researchers to include much diversity of healthy reptiles and amphibians in their projects. Gans (1994) presciently touched on these issues and their real importance, while also pointing out the great multidisciplinary opportunities that exist only on university campuses. Certainly, no zoo maintains physicists, engineers, or chemists on staff, and very few have research facilities fully stocked with the latest technologies and equipment. Gans' (1994) underlying point is that zoos and multiple departments at a university can create a unique synergism that can help move forward important questions in biology and the other natural sciences, as well as effect real progress in conservation.

Research collaborations between zoos and universities are, in general, far too few – at least in the realm of herpetology. As the cultures of these two types of institutions tend to be quite different, collaborations tend to be the products of like-minded individuals from each who have made the effort to collaborate. Formal partnerships between zoos and universities are even rarer. We can point to a few examples, such as a private endowment that links Zoo Atlanta's research staff with the Department of Biological Sciences at the Georgia Institute of Technology, which also supports novel teaching opportunities. A unique model also exists in the Zoological Garden of Tel Aviv University, Israel, in which the zoo is functionally part of the university, with its own dedicated research staff. In contrast, rather than putting a zoo into a university framework, a more familiar model is to maintain academic research staff in a zoo, as in the Institute for Conservation Research at San Diego Zoo or the conservation genetics program at Omaha's Henry Doorly Zoo and Aquarium. A particularly visionary innovation is the incipient Living Earth Collaborative, which brings together the long-running research programs at Saint Louis Zoo, Missouri Botanical Garden, and Washington University. Murphy (2007) provided brief summaries of the traditions and products of herpetological research at major US zoos that involved universities.

In the following case studies, we summarize a few research programs involving reptiles and amphibians that have produced results that extend beyond the traditional boundaries of natural history or pure organismal biology and contribute to a broader perspective of biology and the natural sciences. In other words, it goes without saying that a lot can be learned about the biology of reptiles and amphibians in collaborative efforts involving zoos and universities (see examples above for snakes). Here, we have identified instances where the collaborations have had even broader impacts.

CASE STUDY: AMPHIBIAN DISEASE AND CONSERVATION

The conservation world was rocked in the early 1990s with the slow realization that amphibian populations were declining on multiple continents and, in some cases, resulting in outright extinctions of formerly common species (see review by Collins, Crump, & Lovejoy, 2009). The paradigm shift in this scenario was the absence of a cause for these similar but geographically disjunct events. As academics argued around hypotheses including holes in the ozone layer, UV-B radiation, climate change, and others, conservationists tried to internalize the reality that habitat protection simply was not able to address this mysterious threat. The possibility of a disease being involved began to be discussed (Lips, 1998), but the concept was immediately controversial – indeed, it was outright rejected by some vocal stakeholders – because no pathogen had been identified. Meanwhile, a noteworthy team of zoo and university researchers, working with amphibians that died in zoos and in the field, made the major discovery of a new genus and species of chytrid fungus (*Batrachochytrium dendrobatidis* [*Bd*]) that was pathogenic to amphibians (Longcore, Pessier, & Nichols, 1999). The entire story was recounted by the leading pathologist in the group (Nichols, 2003). A related team, again including zoo and academic authors (Berger et al., 1998), demonstrated the cause–effect relationship between the new chytrid fungus and amphibian declines on two continents. The importance of the discovery of *Bd* cannot be overstated. Knowledge of the pathogen immediately changed the focus of research to the causes of the then-termed "enigmatic declines" of amphibians globally. Well over 1,000 papers on *Bd* have been published since it was discovered (Web of Science, accessed October 7, 2017). The discovery of *Bd* represented a fundamentally new life history among the poorly known chytrid fungi. The precedent for an uncontrollable, emerging infectious disease in wildlife was now established, representing a paradigm shift in conservation action and a notably increased importance of the role of zoos in conservation (Mendelson et al., 2006). Emerging infectious disease as a direct threat to wildlife called for remarkable reforms in wildlife policies worldwide (Lips & Mendelson, 2014; Schloegel et al., 2012). Perhaps most importantly, the precedent of *Bd*-induced decimation of recently robust populations in the wild greatly expedited the response to subsequent epidemics in wildlife (e.g., white-nose syndrome in bats: Blehert et al., 2009). While amphibian biologists were forced to wonder about the cause of their observed declines for nearly a decade before *Bd* was discovered, the offending pathogens in subsequent disastrous scenarios, including an additional chytrid amphibian pathogen (*Batrachochytrium salamandrivorans*: Martel et al., 2013), were identified relatively quickly. This was because the reality of *Bd* had changed the landscape of conservation thinking such that the concept of an unknown, emerging infectious disease in wildlife was no longer dismissed outright as a possible explanation for mysterious declines or die-offs. Appropriately, the American Association for the Advancement of Science recognized the multidisciplinary team that discovered *Bd* with a Golden Goose Award in 2017.

CASE STUDY: PARTHENOGENESIS AND SEX CHROMOSOMES IN SNAKES

The recent news of the discovery of XY sex chromosomes in two ancient snake lineages, the Boidae (*Boa imperator*) and Pythonidae (*Python bivittatus*), upended over 50 years of cytogenetic research on snakes (Gamble et al., 2017; reviewed by Emerson, 2017). Until the publication of the article by Tony Gamble and his colleagues, all extant snake species (>3,500 described) were thought to possess ZW sex chromosomes, despite the fact that evidence for this conclusion in boas and pythons was never substantiated. As we will discuss, the collaborative research by Gamble's team was prompted by events unrelated to the direct study of sex chromosomes. Crucially, research by two of us (GWS, JRM), other academics, zoo personnel, and private citizens (citizen scientists) on facultative parthenogenesis (FP) in snakes ultimately led to the collaborative reanalysis of sex chromosomes in the genera *Boa* and *Python* (Booth & Schuett, 2016). Since the publication of Ohno's (1967) classic book on chromosomes and sex-linked genes, snakes have held the distinction of being a model organism (reviewed by Emerson, 2017). The significance of these new results in snakes cannot be overstated with regard to our understanding of the evolution and function of sex chromosomes of vertebrates (see Bachtrog et al., 2011; Bull, 1983; Charlesworth & Mank, 2010;

Graves, 2013; Mank, Nam, & Ellegren, 2010; Tomaszkiewicz, Medvedev, & Makova, 2017; Vicoso, Emerson, Zektser, Mahajan, & Bachtrog, 2013). Finally, we will discuss the synergistic role of academic–zoo relationships in the scientific exploration of parthenogenesis and sex-chromosome systems in snakes and other reptiles.

The Discovery of Parthenogenesis in Snakes

While parthenogenesis ("virgin birth") is a relatively common mode of clonal reproduction in plants and some animals, it is rare in vertebrates (reviewed by Avise, 2008). In true parthenogenesis, development of offspring occurs in the complete absence of sperm; hence, males are not required for the fertilization of ova. In parthenogenetic reptiles – most cases involve lizards, but one involves a species of tiny blindsnake – males are (sometimes) entirely absent in populations (Cole, 1984; Darevsky, 1958, 1966; Darevsky, Kupriyanova, & Uzzell, 1985). Consequently, under these conditions, parthenogenesis is the obligate mode of reproduction (Neaves & Baumann, 2011). In mammals, successful reproduction is always sexual; the natural occurrence of parthenogenesis resulting in full-term progeny is entirely absent owing to developmental constraints imposed by genomic imprinting, which involves an evolutionary battle between the sexes (reviewed by Haig, 2002; Jaenisch, 1997).

Interestingly, there is a less understood category of parthenogenesis in which organisms that typically reproduce sexually (i.e., union of egg and sperm) also have the capacity for parthenogenetic offspring production. This is termed FP or tychoparthenogenesis (Avise, 2008; Lampert, 2008; Neaves & Baumann, 2011). Today, we know that FP occurs throughout the tree of life, from the simplest organisms to complex ones. In vertebrates, FP was documented extensively in the 1950s in gallinaceous birds, namely domesticated turkeys and chickens (Olsen & Marsden, 1954; reviewed by Olsen, 1975). Around the same time, all-female populations of lizards were being described in Russia and the southwestern United States and were determined to be parthenogens (reviewed by Darevsky, 1958, 1966; Darevsky et al., 1985).

Nearly 50 years after FP was discussed in domestic fowl, this unusual mode of reproduction was discovered in snakes with the aid of a brand-new technology – DNA fingerprinting – developed by Sir Alec Jeffreys (Dubach, Sajewicz, & Pawle, 1997; Schuett et al., 1997). Studies on parthenogenesis in domestic fowl in the 1950s laid the groundwork for interpreting the developmental mechanism of FP in snakes (Schuett et al., 1997; Schuett, Fernandez, Chiszar, & Smith, 1998). Rick Weiss broke the story of snake virgin birth in *The Washington Post* (Weiss, 1997). The article was syndicated and over the next several months it appeared in hundreds of newspapers worldwide – articles also appeared in *New Scientist* (Cohen, 1998), *The Chronicle of Higher Education* (Monaghan, 1997), and other prominent public venues, including the Association of Zoos and Aquariums (AZA) publication *Communiqué* (Demlong & Schuett, 1998).

Subsequent work using increasingly sophisticated molecular and analytical tools (e.g., restriction site-associated DNA sequencing, bioinformatics) not only has documented FP in other snake species (reviewed in Booth & Schuett, 2016), but also in varanid lizards, including the Komodo dragon (Watts et al., 2006), elasmobranch fishes, such as sharks and rays (Chapman, Firchau, & Shivji, 2008; Chapman et al., 2007; Dudgeon, Coulton, Bone, Ovenden, & Thomas, 2017; Feldheim et al., 2010), and non-domesticated birds (Schut, Hemmings, & Birkhead, 2008). The original documentation of FP was restricted to individual females held in captivity and isolated from males, and thus it was labeled as a reproductive error or captive syndrome. However, recent knowledge that FP occurs in nature has shifted the paradigm, and this form of reproduction has been identified in birds, squamate reptiles, and elasmobranch fishes as a feature of vertebrate evolution that cannot be disregarded (Booth et al., 2012, 2014). It made the news (Coles, 2012). Importantly, by establishing the occurrence of FP in snakes, many cases of another phenomenon in snakes – long-term sperm storage (LTSS) – were brought into question (Schuett et al., 1997). Booth and Schuett (2011) reviewed this problem and determined that many reports of LTSS were likely in error and instead supported a model of FP. Although LTSS is common in reptiles and amphibians, Booth and Schuett (2011) offered specific guidelines (e.g., DNA-based evidence) to avoid conflation of these two phenomena in future research. More dogma was upended.

In both of the original papers that reported the discovery of FP in snakes, zoos played a pivotal role. The first paper (Schuett et al., 1997) consisted of eight academics and two curators from the Phoenix Zoo and Toledo Zoo. The second paper (Dubach et al., 1997), in the same journal, was produced by a three-person team, all associated with the Brookfield Zoo. As we will discuss, the paramount role of zoos in the FP story has become more prominent in that additional cases and other discoveries have been reported.

Parthenogenesis and the Discovery of the XY Sex-Determination System in Snakes

> It is very difficult to find a black cat in a dark room.
>
> \- Unknown

To be able to explain the story of how XY chromosomes were discovered in snakes will require a bit more background on FP and a better understanding of how ignorance drives science (*sensu* Firestein, 2012). Providing a plausible developmental mechanism to explain FP in snakes was both puzzling and difficult owing to the fact that only male offspring were produced (Schuett et al., 1998). Models proposed for lizards such as rock lizards and whiptails, especially from well-studied, all-female populations, were not compatible with the data at hand (Cole, 1984; Darevsky, 1958, 1966; Darevsky et al., 1985). As it turned out, we stumbled on a connection between FP in domestic fowl and snakes (see Schuett et al., 1998) that could help us explain the production of only male offspring. In chickens (and other birds) and "advanced" snakes (caenophidians), sperm only bear a Z chromosome and thus are homogametic. In contrast, ova are either Z or W and are heterogametic. When a sperm (Z) fertilizes an ovum bearing a W chromosome, the resulting embryo is ZW and therefore

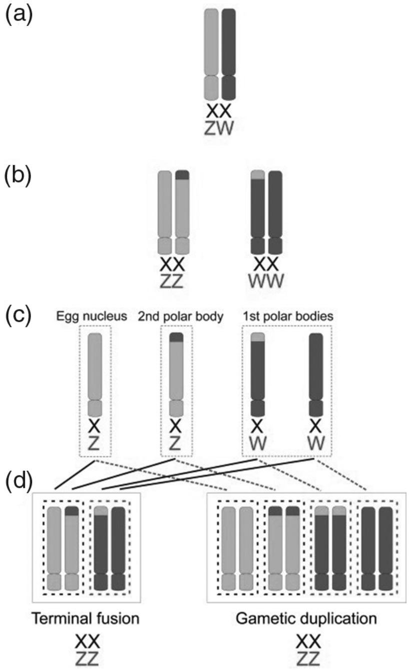

FIGURE 22.1 Automictic parthenogenesis in snakes. Black (upper) letters = boid and pythonid species. Gray (lower) letters = "advanced" (caenophidian) species. (a) Ovum. (b) Meiotic products following DNA replication and recombination during first cell division. (c) Meiotic products following second cell division. (d) Potential chromosomal arrangements following terminal fusion (solid lines) and gametic duplication (dotted lines). The WW arrangements (pairs where both chromosomes are dark gray) are not viable.

Modified from Booth, W. & Schuett, G. W. (2016). The emerging phylogenetic pattern of parthenogenesis in snakes. Biological Journal of the Linnean Society, 118(2), 172–186, by permission of the Linnean Society

female. But when an ovum that bears a Z chromosome is fertilized, it is ZZ and therefore male (reviewed by Avise, 2008; Lampert, 2008). Consequently, as in birds but unlike mammals, female snakes control the sex determination of their progeny (Figure 22.1).

Or at least we thought that was the case until the publication of recent work in 2017.

In 2003, Groot, Bruins, and Breeuwer reported on parthenogenesis in the Burmese python (*Python bivittatus*), a so-called primitive snake species. Unlike the reports by Schuett et al. (1997) and Dubach et al. (1997), where snake progeny were only males, the python fetuses were all females (this problem was discussed by Booth et al., 2014). In 2011, Warren Booth and his colleagues (Booth, Johnson, Moore, Schal, & Vargo, 2011) reported on "virgin birth" in the boa constrictor, a large, primitive snake species of the neotropics. Not surprisingly, it made the news and the internet

(Walker, 2011). Similar to the earlier report on pythons, Booth and colleagues reported that all their boa progeny were females. The observation of all-female offspring was unexpected assuming a ZW sex-determination system, leading the authors to suggest that the female progeny were WW or WO. However, additional cases of FP in other species of boids and pythonids (Booth et al., 2014) and a failure to identify a cytogenetic mechanism leading to WW or WO females cast doubt on this interpretation.

Booth and Schuett (2016) proposed several changes and a daring new hypothesis. First, they proposed partitioning FP in snakes into two categories – type A and type B – based primarily on the sex of the progeny and phylogenetic position (Figure 22.2). Second, they introduced the directionality of heterogamety hypothesis (i.e., the view that evolutionary transitions can occur between XY and ZW sex chromosomes in snake lineages) based on previous cytogenetic research in lizards and other reptiles. Today, next-generation genomic procedures, such as restriction site-associated DNA sequencing, allow researchers to identify transitions among sex-determination systems more readily, especially in those taxa with homomorphic sex chromosomes like boids and pythonids (Becak & Becak, 1969; see Gamble et al., 2015; Gamble & Zarkower, 2014).

Booth, Schuett, and other colleagues teamed up with Tony Gamble and his students and tested this hypothesis using *Boa* and *Python* and the aforementioned genomic procedures. With excitement, evidence for Y-linked DNA markers indicated an XY sex chromosome system and supported the directionality of heterogamety hypothesis. Furthermore, the comparative genomic analysis revealed that *Boa* and *Python* had independently evolved XY systems. This finding put to rest the earlier claim by Booth, Million et al. (2011) that boas produced by parthenogenesis were WW (or even WO). Indeed, the finding of an XY sex-determination system is far more parsimonious and scientifically gratifying than the alternative hypotheses (Figure 22.3). Upending 50 years of dogma, it made the news (Olena, 2017).

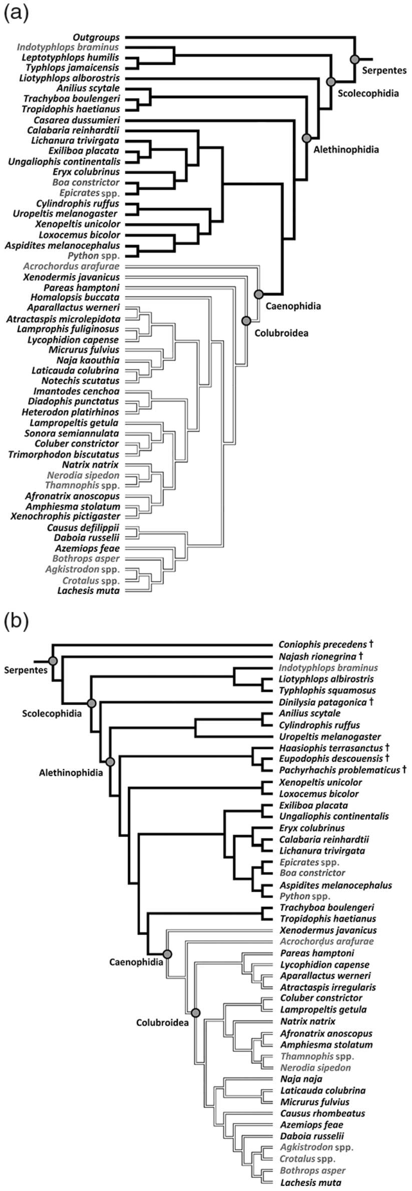

FIGURE 22.2 Phylogeny of parthenogenesis in snakes. (a) A phylogeny constructed from molecular characters (primarily nuclear DNA). (b) A phylogeny constructed from morphological and fossil (extinct taxa) characters. Nodes identify Scolecophidia, Alethinophidia, Caenophidia, and Colubroidea. Where possible, species documented as being parthenogenetic are indicated in brown (obligate), red (facultative, type A), and blue (facultative, type B).

Modified from Booth, W. & Schuett, G. W. (2016). The emerging phylogenetic pattern of parthenogenesis in snakes. Biological Journal of the Linnean Society, 118(2), 172–186, by permission of the Linnean Society. *A black and white version of this figure will appear in some formats. For the color version, please refer to the plate section.*

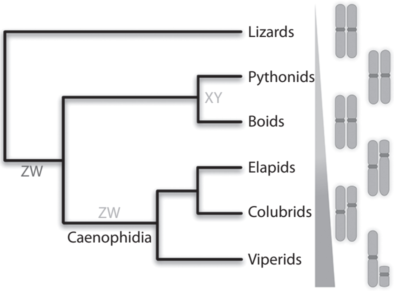

FIGURE 22.3 Phylogenetic pattern of sex chromosome evolution in snakes. The phylogeny shows the relationships between selected major groups of snakes and the chromosome morphology of what is typically the fourth-largest pair of chromosomes in male snakes, which is homologous to chromosome 6 in the green anole (*Anolis carolinensis*). The chromosome delineations denote variation in chromosome size and centromere location. The triangle indicates the accepted continuum model for sex chromosome evolution from their autosomal ancestors; the narrow end represents no differentiation between Z and W and the wider end means substantial differentiation. The dark gray ZW is the timing of acquisition of ZW chromosomes under the accepted model and the light gray XY and ZW denote the alternative model recently proposed by Tony Gamble and his team (Gamble et al., 2017).

Reprinted from *Current Biology*, 27, J. J. Emerson, Evolution: A paradigm shift in snake sex chromosome genetics, R800–R803, Copyright 2017, with permission from Elsevier

Implications of XY Sex-Determination in Boids and Pythonids

Robust evidence provided by Gamble and his team squarely puts the importance of the discovery of XY chromosomes in boids and pythonids at center stage for future research. As Emerson (2017) remarked, "If [corroborated], these results will transform the field of sex chromosome evolution and genetics in snakes [. . .] the age of the ZW chromosome pair in caenophidians could be potentially much younger than previously thought [. . .] the prospect of rapid sex chromosome transitions in snakes will ensure that snakes remain a model system for studying the evolution of sex chromosomes."

Finally, the discoveries discussed in this section did not have their origins based on a traditional hypothesis testing framework.

Rather, they were more or less "accidents" that were unanticipated, occurring out of the blue or while other research topics were being explored. Stuart Firestein, a neuroscientist and chair of the Department of Biology at Columbia University, has written a short yet compelling book that provides the best explanation of this kind of research – ignorance as a powerful driver of science (Firestein, 2012). Odd as it may seem, this perspective will alter your view of how science operates in the real world. We encourage students, scientists, zoo personnel, and laypersons interested in the true processes of scientific discovery to dig into these pages. Another must-read gold standard is Thomas Kuhn's book on the structure of scientific revolutions (Kuhn, 1996).

CASE STUDY: PLAY IN REPTILES

The Concept of Play

In highly social and intelligent animals, play has been acknowledged for centuries by philosophers and scientists as a common and normal behavior (Darwin, 1874). In mammals, particularly carnivores (e.g., sea lions, otters, meerkats, wolves, bears), cetaceans (dolphins, whales), primates (monkeys, great apes), and equids (horses, zebra), play behavior is commonly exhibited in nature and captivity, especially in zoos and aquariums. Play behavior in mammals is a great attraction enjoyed by the public. Allowing captive animals to engage in social and private play is not only entertaining, but also contributes to enrichment (e.g., psychosocial and physiological well-being) by ameliorating stresses (Moberg, 1985; Serres & Delfour, 2017). Furthermore, because play is natural and relevant to humans, it is rarely a cause for controversy as it pertains to animal safety. Zoos and aquariums often provide optimal viewing situations (e.g., large glass stations, elevated platforms, digital signage kiosks, and bleachers) where the public is encouraged to witness play behavior. Many animals, especially dogs, horses, cetaceans, and carnivores, are especially amenable to human cues during public performances where specific behaviors, including play, are encouraged, rewarded, and enriching (Mikloski & Soproni, 2006; Schuetz, Farmer, & Krueger, 2017; Westlund, 2014).

Until recently, however, play in reptiles was considered by most behaviorists as a foolish notion and something of a fable, a *non contingat*. Despite the skepticism, some researchers, such as Gordon Burghardt (Chapter 21, this volume), thankfully followed a different path and explored play in reptiles and other taxa with a fresh viewpoint. The old scientific approaches that placed severe limits on what "cold-blooded" vertebrates can do or experience are dropping out of vogue, while fresh and innovative perspectives are being embraced and studied, such as social behaviors, personality/temperament, and play (Burghardt, Dinets, & Murphy, 2014; Dinets, 2015; Doody, Burghardt, & Dinets, 2013; Schuett et al., 2016; Waters, Bowers, & Burghardt, 2017). Today, with the inclusion of a greater diversity of vertebrate (and invertebrate) taxa – fishes, amphibians, reptiles, spiders, and octopuses – research on play behavior is undergoing a paradigm shift in the broader disciplines of cognition and comparative behavior (Burghardt, 2005, 2015; Burghardt et al., 2014; Hopper, 2017; Pruitt, Burghardt, & Riechert, 2012; Zylinski, 2015). Zoos can provide an excellent *ex situ* environment for the study of play and other behaviors owing to the availability of space, diversity of species, and, in some cases, the capability of maintaining large numbers of individuals for long periods. Few academic institutions are capable of maintaining this type of rich diversity, especially in reptiles, where it concerns giant snakes, tortoises, and crocodilians. Indeed, research on play behavior lends itself quite well to the establishment of long-term academic–zoo partnerships (reviewed in Hopper, 2017).

Defining Play

> Play (v.) – engagement in an activity for enjoyment and recreation, rather than serious or practical purposes, especially by children.
>
> – Modified from Oxford Dictionaries

Gordon Burghardt, one of the pioneers and leaders of play behavior in nonhuman animals, especially reptiles and other ectothermic ("cold-blooded") vertebrates, provides us with five main criteria that must be met for a behavior to be characterized or defined as play. Such criteria are necessary to avoid conflation with other types of behavior and to be testable within formal, hypothesis-based inquiry (Burghardt, 2011; Miller, 2017).

The following five criteria of play are modified slightly from Burghardt (2005):

(a) The behavior is not fully functional in the form, or context, in which it is expressed.
(b) The behavior is spontaneous, voluntary, intentional, pleasurable, rewarding, reinforcing, and executed for its own sake.
(c) The behavior is incomplete, exaggerated, awkward, and or precocious.
(d) The behavior is repeated in a similar manner, but it is not rigidly stereotyped.
(e) The behavior is initiated when an individual is replete (well fed), healthy, free from stress, and in a state of relaxation.

Burghardt (2005, p. 82) provided a brief definition of play: "Play is a repeated, incompletely functional behavior differing from more serious versions structurally, contextually, or ontogenetically, and initiated voluntarily when the animal is in a relaxed or low stress setting."

Categories of Play

Play behavior carried out by nonhuman animals can be classified into three main categories, which are not necessarily mutually exclusive (see Burghardt, 1999, 2011; Dinets, 2015; *sensu Fagan*, 1981). In fact, all three types of play may be witnessed in a single observation episode. These categories are:

(a) Locomotor play
(b) Object play
(c) Social play

In locomotor play, behaviors such as running, leaping, gliding, sliding, surfing, and brachiating are noteworthy. Who has not been amused at watching playful otters sliding down a worn embankment, or watching spider monkeys swing from branch to branch (brachiating) and from tree to tree? Object play involves the manipulating, pulling, pushing, and/or chewing of objects. Common examples include chasing balls and chewing on sticks. Social play involves same-species activities, and common behaviors include rough and tumble, chasing, nipping, and vocalizing. Wolf pups chasing and tumbling with each other is a heart-warming scene (Bekoff, 2007). Another category of so-called social play that is gaining attention in zoos is "heterospecific play," whereby by humans or different species (e.g., cheetah and dog) are involved and act as a social surrogate for enrichment (www.youtube.com/watch?v=Ndlf5_L5gsE).

Play Behavior in Reptiles

> Ignorance more frequently begets confidence than knowledge.
>
> – *Charles Darwin*

Until recently, the topic of play in reptiles was not only vastly understudied (actually, it was essentially absent from the literature; Beach, 1945), but anyone who investigated it was almost certainly shunned by colleagues and treated as a charlatan of real science (Burghardt, 2005). Today, it is a viable and legitimate topic in the study of vertebrate behavior and cognition (Bateson & Martin, 2013; Burghardt et al., 2002; Dinets, 2015; Doody et al., 2013; Graham & Burghardt, 2010; Waters, Bowers, & Burghardt, 2017).

As recently as the early 1980s, Fagan (1981) could locate only two examples (anecdotes) of reptile play – object play in a captive Komodo dragon, *Varanus komodoensis* (Hill, 1946), and a field observation of an American alligator (*Alligator mississippiensis*) repeatedly attracted to and snapping at water dripping from a spout (Lazell & Spitzer, 1977; see review in Dinets, 2015). Below, we provide examples of play behavior and studies on reptiles since Fagan's (1981) review.

Examples of Play in Reptiles

Table 22.1 provides examples of play behavior in reptiles since the review by Fagan (1981). To date, object play is the most commonly reported category of play in this vertebrate group, though all three categories (object, locomotor, and social) of play are reported in crocodilians (see review by Dinets, 2015).

Implications of Studying Play in Reptiles

For certain species of reptiles, such as adult crocodilians, giant tortoises, large lizards (e.g., Komodo dragons), giant pythons, and highly venomous snakes (e.g., king cobras), zoos and similar venues have been a primary source for the detection and study of play behavior. Furthermore, many of these taxa are rare and no longer commercially available or have other restrictions with regard to use. Most academic settings, such as universities, are largely unequipped to provide adequate long-term (years, decades) care for these kinds of vertebrates due to inadequate facilities and personnel. On the other hand, most zoos lack the resources required for research, including individuals with academic training. Consequently, for noninvasive research on play and cognition in reptiles, zoo–academic relationships provide the best of both worlds in an innovative and synergistic environment (Burghardt, 2005, 2013; Dinets, 2015; Hopper, 2017).

CASE STUDY: SNAKES AS LIVING BIOMODELS

Collins (2002) encouraged academics to embrace multidisciplinary approaches as they became more common, and the approach brings breadth and depth to the subject matter, especially, as technologies advance so rapidly. Bio-inspired design projects may be seen as multidisciplinary efforts and, indeed, the term and the field (biomimicry) have been popular (130,000 records; GoogleScholar, October 19, 2017). While these efforts arise from collaborations across disciplines, the results are usually singular, namely a new or improved device that has been optimized using some biological model. These efforts, however, can be reciprocally informative if the device is then used to inform

Table 22.1 *Examples of play behavior in reptiles.*

Taxon	Play category	Description	References
Crocodylia			
Alligator mississippiensis	Locomotor	Sliding	Burghardt (2005); Dinets (2015)
Caiman latirostris	Locomotor	Surfing	Dinets (2015)
Crocodylus porosus	Locomotor	Surfing	Dinets (2015)
Crocodylus rhombifer	Object	Ball	Dinets (2015)
Melanosuchus niger	Social	Chasing	Dinets (2015)
Paleosuchus palpebrosus	Object	Showering	Dinets (2015)
Testudines			
Pseudemys nelsoni	Social	Courtship	Kramer and Burghardt (1998)
Trionyx triunguis	Object	Basketball	Burghardt (2005); Burghardt et al. (1996)
Centrochelys sulcata	Object	Soccer ball	YouTube (2013)[a]
Squamata			
Sauria			
Chondrodactylus turneri	Object	Collar	Barabanov et al. (2015)[b]
Varanus komodoensis	Object	Toys	Burghardt (2005); Burghardt et al. (2002)
Serpentes			
Pituophis catenifer	Locomotor	Cord	Burghardt (2005)[c]

[a] www.youtube.com/watch?v=3lhtzgAdm58&t=19s

[b] Observations were made during a 30-day orbital (zero-gravity) experiment on the unmanned spacecraft BION-M No. 1.

[c] This observation is questionable as play; play behavior in snakes appears to be rare and difficult to document (per Burghardt, 2005).

Note: Examples of play in reptiles since the review by Fagan (1981). This list is not exhaustive.

and advance the study of the biological model. In the example here, a robot's performance was improved by a model system drawn from nature (a snake), and then the robot was used to test the locomotor results of optimal and nonoptimal performance, observed and not observed, across a variety of species of snakes.

A series of papers (Astley et al., 2015; Gong et al., 2015; Marvi et al., 2014) were produced when a team comprising robotics engineers from Carnegie Mellon University, physicists from Georgia Tech, and biologists from Zoo Atlanta collaborated to achieve the major goal of expanding the capabilities of an existing snake-based robot to include functionality on sand substrates. The biology model involved was the sidewinder rattlesnake (*Crotalus cerastes*). Once the locomotion of the snake on different slope angles of sand was fully quantified, the robot was then programmed to mimic it and proceeded to successfully navigate across sand on every possible slope. However, rather than sticking to a singular, one-directional, bio-inspired design model, the teams invoked a comparative approach to quantify "failure to sidewind" across many species of snakes, including rattlesnakes closely related to the sidewinder. Programming the robot to perform the same motions illustrated exactly how these other snakes were failing (e.g., too much lift or too much body contact). Plotting the robot's performances identified that the sidewinder is, in fact, optimal in its performance on sand; that is to say, the body movements observed in sidewinders represent the most optimal and efficient way to move on sand on any given incline. Quantitatively different body motions would lead to reduced efficiency or failure, and these behaviors were never observed across many hundreds of hours of sidewinder trials. A similar study, involving parts of the same team, had previously built robots capable of rectilinear motion, based on analyses of large pythons and boas in the zoo's collection (Marvi, Bridges, & Hu, 2013).

In a related set of experiments, our collaborative group used the western shovel-nosed snake (*Chionactis occipitalis*) to investigate the functionality of the elongate snake body form in subsurface swimming through granular media (i.e., sand or plastic beads). The species co-occurs with the sidewinder across much of the Mojave and Sonoran deserts in North America. In fact, these two species appear to be the only snakes living in the sandiest areas of their ranges. The shovel-nosed snake moves readily across the surface of the sand, and our team is currently investigating those locomotor behaviors. Recently, we published the results of studies of their subsurface behaviors (Sharpe et al., 2015). Early studies of these snakes in captivity (e.g., Norris & Kavanau, 1966) were limited by their ability to observe or measure activities below opaque sand. We used high-speed x-ray imaging of subsurface kinematics of snakes with small lead markers affixed to the body, allowing real-time and slow-motion quantification of their behaviors. Experimental data from the shovel-nosed snake were then compared to previous studies (e.g., Ding, Sharpe, Masse, & Goldman, 2012) of another well-known sand-swimming lizard, the sandfish skink (*Scincus scincus*). The results showed that the snake moved under the surface with much greater efficiency (measured as cost of transport) by virtue of the lower frictional properties of its skin and largely because of the serpentine body form comprising a long, thin shape in which a very high number of vertebral joints allows more bends of greater curvature to be made along the length of body. Inasmuch as granular media can exist in a fluidized state (e.g., fine sand pouring downward in an antique hourglass), the snake's body follows the path of the head through a self-made, but temporary, tube that is lined by fluidized sand at the interface with the skin. Rather than using a robot as a non-biological, manipulable proxy for simulation studies, these studies used steel rods in a glass bead granular media to test drag forces created by non-serpentine "behaviors."

As in the sidewinder and other case studies presented here, these studies produced new knowledge about the biology of the focal species, but also contributed on a broader biological scale. Sharpe et al. (2015) summarized the potential broader implications of their findings, relating the functionality and efficiency of elongation and increased vertebral number to the repeated evolution of those traits in squamate reptiles and indeed perhaps to the origin of snakes specifically. The story here pertains to much more than a single peculiar species of snake in the American deserts.

CASE STUDY: BIOMEDICAL GENOMICS

Reptiles provide a spectrum of cardiac morphologies that are central to a truly phylogenetic perspective of the evolution of the amniote cardiovascular system. This spectrum includes the primitive, three-chambered morphology seen in many small lizards to the fully separated, four-chamber morphologies in crocodilians and birds. Intermediate morphologies are known in turtles and varanid lizards. The latter group of lizards are also of especial interest because of their elevated metabolic rates, which are unmatched across non-avian reptiles and approach those of mammals. Associated with this near-mammalian physiology in varanid lizards is a heart morphology in which an enlarged muscular ridge results in a functionally divided heart with no mixing of venous and arterial blood. In effect, these lizards have the functionality of a fully separated, four-chambered heart that is technically a partially divided, three-chambered organ (Burggren & Johansen, 1982). This derived cardiac morphology – and, more specifically, the genomic basis for its development – is of exceptional interest in tracking the evolution of vertebrate cardiac functionality (Koshiba-Takeuchi et al., 2009).

There is more of interest here, however, than just the basic biology of reptilian cardiovascular systems. The spectrum of cardiac morphologies among reptiles of course belies the spectrum of genetic sequences and expressions that drive the morphogenesis of these cardiac structures. Benoit Bruneau at the University of California, San Francisco studies cardiac morphogenesis in general as well as congenital cardiac malformations in humans. He and his lab identified varanid lizards, and the Komodo dragon (*Varanus komodoensis*) specifically, as a key evolutionary and developmental model for understanding different genetic regulatory networks that can produce a non-four-chambered heart that can effectively isolate oxygenated and deoxygenated blood flows. Of especial collateral interest here is the work in the Bruneau lab on congenital heart disease in humans. In humans, irregularities in the genetic and molecular pathways of cardiac morphogenesis result in malformed hearts. In the most basic terms, a goal here may be the comparison of the genome that produces a normal heart in a varanid lizard with that which produces a superficially similar heart that is not normal in a human. The entire human genome has been sequenced so, to achieve the goal above, the Bruneau lab is working on recovering the complete genome of the Komodo dragon. To date, the genome of only one species of lizard is known (green anole, *Anolis carolinensis*), and this species is not as informative as would be a varanid lizard in terms of the broader aspects of cardiac structure and functionality.

The raw materials necessary to construct the complete genome of the Komodo dragon are a few milliliters of fresh blood from a single lizard. At the outset, the Bruneau lab was frustrated because their initial queries to the multiple AZA zoos that have this species were met with no response or rejection with no further consideration. At least one zoo told them that "Our animals are not to be used for research." Eventually, a colleague in academia referred them to Zoo Atlanta, where a subsample of a routine health screen blood draw on a dragon was shipped to the Bruneau lab to initiate the project. In such a simple manner, the collections of a zoo can make possible truly cross-disciplinary research programs – in this case, one with considerable implications for evolutionary and developmental biology and herpetology, and with applications to human health.

CONCLUSIONS

The role of academic–zoo relationships in the discoveries described above cannot be overstated. It is fair to say that one without the other would have greatly impeded the research. Furthermore, a citizen science approach was also used for information and data acquisition, using Facebook and other social media platforms. Private individuals were encouraged to participate in the research (Facultative Parthenogenesis in Reptilia, 2014). The importance of zoo–academic partnerships and the role of citizen science in future studies of these phenomena in snakes and other animals cannot be overstated. Rarely will universities permit the housing of dangerously venomous snakes or giant constrictors, yet these are the precise taxa that opened the doors to the discoveries of FP and sex chromosomes and were the models to inform robotic design. Oppositely, with few exceptions, most zoos do not have the economic engines to obtain and maintain next-generation sequencing equipment, high-speed 3-D camera imaging systems, or sub-surface x-ray and other rare technologies, nor the technicians and scientists to oversee their use and interpret results.

However, collaborations are two-way streets. Zoos must be willing to consider research requests from the academy, including those that may be of little interest to the zoo and may have no direct applications to improving animal welfare. The knee-jerk attitude of "our animals are not to be used for research" of some institutions renders moot every point made in this chapter. However, a required door is simply some form of internal scientific review committee at the zoo that screens inquiries that are too invasive. An open ear to research requests is not an open door. Consequently, by eliminating barriers and facilitating cooperation, we envision a much broader landscape of zoo–academic partnerships. The research we describe was noninvasive and acceptable in both academic and zoo environments. Importantly, such research has captivated the public across numerous media events and improved relations (academia–zoos–public) in a cost-effective manner, truly a trifecta of accomplishments.

ACKNOWLEDGMENTS

Warren Booth, Root Gorelick, and John Avise were always available for discussions on clonality and parthenogenesis. Gordon Burghardt was helpful in discussing play in reptiles. One of us (GWS) thanks all of the coauthors on the parthenogenetic and sex chromosome papers (see Schuett & Booth, 2017). Other helpful discussions and comments were provided by Dan Goldman, Karen Lips, Lori Perkins, Rebecca Snyder, Bruneau Benoit, Sam Rivera, Charles Knapp, Catherine Malone, Brian Kubicki, Luis Coloma, Tara Stoinski, Julia Paull, Hayley Murphy, James Murphy, Jennifer Pramuk, Andrew Odum, Terry Maple, Robert Hill, James Collins, Robert Mendyk, Ben Minteer, and George Rabb. Olivia Malice and Caroline Long were exceptionally helpful in final formatting, copy editing, and chasing elusive references.

REFERENCES

Astley, H. C., Gong, C., Travers, M., Serrano, M. M., Vela, P. A., Choset, H., … Goldman, D. I. (2015). Modulation of orthogonal body waves enables high maneuverability in sidewinding locomotion. *Proceedings of the National Academy of Sciences*, *112*, 6200–6205.

Attractions Magazine (2012). Cheetah and dog friends celebrate anniversary together at Busch Gardens Tampa [Video File]. Retrieved from www.youtube.com/watch?v=Ndlf5_L5gsE.

Auffenberg, W. (1981). The *Behavioral Ecology of the Komodo Monitor*. Gainsville, FL: University Press of Florida.

Avise, J. C. (2008). *Clonality: The Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals*. New York: Oxford University Press.

Bachtrog, D., Kirkpatrick, M. Mank, J. E., McDaniel, S. F., Pires, J. S., Rice, W. R., & Valenzuela, N. (2011). Are all sex chromosomes created equal? *Trend in Genetics*, *27*, 350–357.

Barabanov, V., Gulimova, V., Berdiev, R., & Saveliev, S. (2015). Object play in thick-toed geckos during a space experiment. *Journal of Ethology*, *33*, 109–115.

Bateson, P. & Martin, P. (2013). *Play, Playfulness, Creativity, and Innovation*. Cambridge: Cambridge University Press.

Beach, F. A. (1945). Current concepts of play in animals. *American Naturalist*, *79*, 523–541.

Becak, W. & Becak, M. L. (1969). Cytotaxonomy and chromosomal evolution in Serpentes. *Cytogenetics*, *8*, 247–262.

Bekoff, M. (2007). *The Emotional Lives of Animals*. Novato, CA: New World Library.

Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., … Hines, H. B. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences*, *95*, 9031–9036.

Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., … Okoniewski, J. C. (2009). Bat white-nose syndrome: An emerging fungal pathogen? *Science*, *323*, 227–227.

Booth, W. & Schuett, G. W. (2011). Molecular genetic evidence for alternative reproductive strategies in North American pitvipers (Serpentes, Viperidae): Long-term sperm storage and facultative parthenogenesis. *Biological Journal of the Linnean Society*, *104*, 934–942.

Booth, W. & Schuett, G. W. (2016). The emerging phylogenetic pattern of parthenogenesis in snakes. *Biological Journal of the Linnean Society*, *118*, 172–186.

Booth, W., Johnson, D. H., Moore, S., Schal, C., & Vargo, E. L. (2011). Evidence for viable, non-clonal but fatherless boa constrictors. *Biology Letters*, *7*, 257–260.

Booth, W., Million, L., Reynolds, R. G., Burghardt, G. M., Vargo, E. L., Schal, C., … Schuett, G. W. (2011). Consecutive virgin births in the New World boid snake, the Colombian Rainbow Boa, *Epicrates maurus*. *Journal of Heredity*, *102*, 759–763.

Booth, W, Schuett, G. W., Ridgway, A., Buxton, D., Castoe, T. A., Bastone, G., … McMahan, C. W. (2014). New insights on facultative parthenogenesis in pythons. *Biological Journal of the Linnean Society*, *112*, 461–468.

Booth, W., Smith, C. F., Eskridge, P. H., Hoss, S. K., Mendelson III, J. R., & Schuett, G. W. (2012). Facultative parthenogenesis discovered in wild vertebrates. *Biology Letters*, *8*, 983–985.

Bull, J. J. (1983). *Evolution of Sex Determining Mechanisms*. Menlo Park, CA: Benjamin Cummings.

Burghardt, G. M. (1999). Play. In G. Greenberg & M. M. Haraway (Eds.), *Comparative Psychology: A Handbook* (pp. 725–735). New York: Garland Publishing Co.

Burghardt, G. M. (2005). *The Genesis of Animal Play: Testing the Limits*. Cambridge, MA: MIT Press.

Burghardt, G. M. (2011). Defining and recognizing play. In A. D. Pellegrini (Ed.), *The Oxford Handbook of the Development of Play* (pp. 9–18). New York: Oxford University Press.

Burghardt, G. M. (2013). Environmental enrichment and cognitive complexities in reptiles and amphibians: Concepts, review and implications for captive populations. *Applied Animal Behaviour Science*, *147*, 286–298.

Burghardt, G. M. (2014). A brief glimpse at the long evolutionary history of play. *Animal Behavior and Cognition*, *1*, 90–98.

Burghardt, G. M. (2015). Play in fishes, frogs and reptiles. *Current Biology*, *25*, R9–R10.

Burghardt, G. M., Chiszar, D., Murphy, J. B., Romano, J., Walsh, T., & Manrod, J. (2002). Behavioral complexity, behavioral development, and play. In J. B. Murphy, C. Ciofi, C. Panouse, & T. Walsh (Eds.), *Komodo Dragons: Biology and Conservation* (pp. 78–118). Washington, DC: Smithsonian Institution Press.

Burghardt, G. M., Dinets, V., & Murphy, J. B. (2014). Highly repetitive object play in a cichlid fish (*Tropheus duboisi*). *Ethology*, *121*, 38–44.

Burghardt, G. M., Ward, B., & Rosscoe, R. (1996). Problem of reptile play: Environmental enrichment and play behavior in a captive Nile soft-shelled turtle (*Trionyx triunguis*). *Zoo Biology*, *15*, 223–228.

Burggren, W. & Johansen, K., (1982). Ventricular haemodynamics in the monitor lizard *Varanus exanthematicus*: Pulmonary and systemic pressure separation. *Journal of Experimental Biology*, *96*, 343–354.

Card, W. C., Roberts, D. T., & Odum, R. A. (1998). Does zoo herpetology have a future? *Zoo Biology*, *17*, 453–462.

Chapman, D. D., Firchau, B., & Shivji, M. S. (2008). Parthenogenesis in a large-bodied requiem shark, the blacktip *Carcharhinus limbatus*. *Journal of Fish Biology*, *73*, 1473–1477.

Chapman, D. D., Shivji, M. S., Louis, E., Sommer, J., Fletcher, H., & Prödohl, P. A. (2007). Virgin birth in a hammerhead shark. *Biology Letters*, *3*, 425–427.

Charlesworth, D. & Mank, J. E. (2010) The birds and the bees and the flowers and the trees: Lessons from genetic mapping of sex determination in plants and animals. *Genetics*, *186*, 9–31.

Cohen, P. (1998). Like a virgin. *New Scientist*, *160*, 36–39.

Cole, C. J. (1984). Unisexual lizards, *Scientific American*, *50*, 94–101.

Coles, J. (2012). Virgin births discovered in wild snakes. *BBC Nature* (September 12, 2012). Retrieved from www.bbc.co.uk/nature/19555550.

Collins, J. P. (2002). May you live in interesting times: Using multidisciplinary and interdisciplinary programs to cope with change in the life sciences. *BioScience*, *52*, 75–83.

Collins, J. P., Crump, M. L., & Lovejoy III, T. E. (2009). *Extinction in Our Times: Global Amphibian Decline*. New York: Oxford University Press.

Darwin, C. (1874). *The Descent of Man*, 2nd edn. London: Murray.

Darevsky, I. S. (1958). Natural parthenogenesis in certain subspecies of the rock-lizard *Lacerta saxicola* Eversmann. *Doklady Academii Nauk SSSR*, *122*, 730–732.

Darevsky, I. S. (1966). Natural parthenogenesis in a polymorphic group of Caucasian rock lizards related to *Lacerta saxicola* Eversmann. *Journal of the Ohio Herpetological Society*, *5*, 115–152.

Darevsky, I. S., Kupriyanova, L. A., & Uzzell, T. (1985). Parthenogenesis in reptiles. In C. Gans & D. F. Billett (Eds.), *Biology of the Reptilia, 15* (pp. 413–526). New York: John Wiley & Sons.

Demlong, M. & Schuett, G. (1998). The Phoenix Zoo announces birth. *Communiqué*, July, 35.

Deufel, A. & Cundall, D. (2003). Feeding in *Atractaspis* (Serpentes: Atractaspididae): A study in conflicting functional constraints. *Zoology*, *106*, 43–61.

Dinets, V. (2015). Play behavior in crocodilians. *Animal Behavior and Cognition*, *2*, 49–55.

Ding, Y., Sharpe, S. S., Masse, A., & Goldman, D. I. (2012). Mechanics of undulatory swimming in a frictional fluid. *PLoS Computational Biology*, *8*, e1002810.

Doody, J. S., Burghardt, G. M., & Dinets, V. (2013). Breaking the social–non-social dichotomy: A role for reptiles in vertebrate social behavior research? *Ethology*, *199*, 1–9.

Dubach, J., Sajewicz, A., & Pawley, R. (1997). Parthenogenesis in the Arafuran filesnake (*Acrochordus arafurae*). *Herpetological Natural History*, 5, 11–18.

Dudgeon, C. L., Coulton, L., Bone, R., Ovenden, J. R., & Thomas, S. (2017). Switch from sexual to parthenogenetic reproduction in a zebra shark. *Scientific Reports*, 7, 40537.

Emerson, J. J. (2017). Evolution: A paradigm shift in snake sex chromosome genetics. *Current Biology*, *27*, R800–R803.

Facultative Parthenogenesis in Reptilia (2014). Faculative Parthenogensis in Reptilia Facebook group page. Retrieved from www.facebook.com/groups/463481383787022.

Fagan, R. (1981). *Animal Play Behavior*. New York: Oxford University Press.

Feldheim, K. A., Chapman, D. D., Sweet, D., Fitzpatrick, S., Prodöhl, P. A., Shivji, M. S., & Snowden, B. (2010). Shark virgin birth produces multiple viable offspring. *Journal of Heredity*, *101*, 374–377.

Firestein, S. (2012). *Ignorance: How It Drives Science*. New York: Oxford University Press.

Gamble, T. & Zarkower, D. (2014). Identification of sex-specific molecular markers using restriction site associated DNA sequencing. *Molecular Ecology Resources*, *14*, 902–913.

Gamble, T., Castoe, T. A., Nielsen, S. V., Banks, J. L., Jaison, L., Card, D. C., ... Booth, W. (2017). The discovery of XY sex chromosomes in *Boa* and *Python*. *Current Biology*, *27*, 2148–2153.

Gamble, T., Coryell. J., Ezaz, T., Lynch, J., Scantlebury, D. P., & Zarkower D. (2015). Restriction site-associated DNA sequencing (RAD-seq) reveals an extraordinary number of transitions among gecko sex-determining systems. *Molecular Biology and Evolution*, *32*, 1296–1309.

Gans, C. (1994). Directions for future research at academic institutions. In J. B. Murphy, K. Adler, & J. T. Collins (Eds.), *Captive Management and Conservation of Amphibians and Reptiles, Contributions to Herpetology*, Vol. 1 (pp. 391–395). Ithaca, NY: Society for the Study of Amphibians and Reptiles.

Gong, C., Travers, M., Astley, H., Li, L., Mendelson, J., Hu, D., . . . Choset, H. (2015). Kinematic gait synthesis for snake robots. *The International Journal of Robotics Research*, *34*, 1–14.

Graham, K. L. & Burghardt, G. M. (2010). Current perspectives on the biological study of play: Signs of progress. *Quarterly Review of Biology*, *85*, 393–418.

Greene, H. W. (1976). Scale overlap, a directional sign stimulus for prey ingestion by ophiophagous snakes. *Ethology*, *41*, 113–120.

Greene, H. W. & Burghardt, G. M. (1978). Behavior and phylogeny: Constriction in ancient and modern snakes. *Science*, *200*, 74–77.

Graves, J. A. M. (2013). How to evolve new vertebrate sex determining genes. *Developmental Dynamics*, *242*, 354–359.

Groot, T. V. M., Bruins, E., & Breeuwer, J. A. J. (2003). Molecular genetic evidence for parthenogenesis in the Burmese python, *Python molurus bivittatus*. *Heredity*, *90*, 130–135.

Haig, D. (2002). *Genomic Imprinting and Kinship*. New Brunswick, NJ: Rutgers University Press.

Harvey, P. H. & Pagel, M. D. (1991). *The Comparative Method in Evolutionary Biology.* Oxford: Oxford University Press.

Hill, C. (1946). Playtime at the zoo. *ZOO-LIFE*, *1*, 24–26.

Hill, R. L., Mendelson III, J. R., & Stabile, J. L. (2015). Direct observation and review of herbivory in Sirenidae (Amphibia: Caudata). *The Southeastern Naturalist*, *14*, N5–N9.

Hopper, L. M. (2017). Cognitive research in zoos. *Current Opinions in Behavioral Sciences 16*, 100–110.

Jaenisch, R. (1997). DNA methylation: Why bother? *Trends in Genetics*, *13*, 323–329.

Koshiba-Takeuchi, K. T., Mori, A. D., Kaynak, B. L., Cebra-Thomas, J., Sukonnik, T., Georges, R. O., . . . Bruneau, B.G. (2009). Reptilian heart development and the molecular basis of cardiac chamber evolution. *Nature*, *461*, 95–98.

Kramer, M. & Burghardt, G. M. (1998). Precocious courtship and play in emydid turtles. *Ethology*, *104*, 38–56.

Krogh, A. (1929). The progress of physiology. *American Journal of Physiology – Legacy Content*, *90*, 243–251.

Kuhn, T. S. (1996). *The Structure of Scientific Revolutions*, 3rd edn. Chicago, IL: University of Chicago Press.

Lampert, K. P. (2008). Facultative parthenogenesis in vertebrates: Reproductive error or chance? *Sexual Development*, *2*, 290–301.

Lazell, J. D. & Spitzer, N. C. (1977). Apparent play in an American alligator. *Copeia*, *1977*, 18.

Lindstedt, S. (2014). Krogh 1929 or The Krogh Principle. *Journal of Experimental Biology*, *217*, 1640–1641.

Lips, K. R. (1998). Decline of a tropical montane amphibian fauna. *Conservation Biology*, *12*, 106–117.

Lips, K. R. & Mendelson, J. R., III (2014). Stopping the next amphibian apocalypse. *New York Times*. November 14, 2014.

Longcore, J. E., Pessier, A. P., & Nichols, D. K. (1999). *Batrachochytrium dendrobatidis gen. et sp. nov.:* A chytrid pathogenic to amphibians. *Mycologia*, *1999*, 219–227.

Mank, J. E., Nam, K., & Ellegren, H. (2010). Faster Z evolution is predominantly due to genetic drift. *Molecular Biology and Evolution*, *27*, 661–670.

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M. C., . . . Pasmans, F. (2013). *Batrachochytrium salamandrivorans sp. nov.* causes lethal chytridiomycosis in amphibians. *Proceedings of the National Academy of Sciences*, *110*, 15325–15329.

Marvi, H., Bridges, J., & Hu, D.L. (2013). Snakes mimic earthworms: Propulsion using rectilinear travelling waves. *Journal of the Royal Society Interface*, *10*, 20130188.

Marvi, H., Gong, C., Gravish, N., Astley, H., Hatton, R. L., Mendelson, J. R., III, . . . Goldman, D. I. (2014). Sidewinding with minimal slip: Snake and robot ascent of sandy slopes. *Science*, *346*, 224–229.

Medvedev, M. & Makova, K. D. (2017). Y and W chromosome assemblies: Approaches and discoveries. *Trends in Genetics*, *33*, 266–282.

Mendelson, J. R., III, Lips, K. R., Gagliardo, R. W., Rabb, G. B., Collins, J. P., Diffendorfer, J. E., . . . Brodie, E. D., Jr. (2006). Policy forum: Confronting amphibian declines and extinctions. *Science*, *313*, 48.

Mikloski, A. & Soproni, A. (2006). A comparative analysis of animals' understanding of the human pointing gesture. *Animal Cognition*, *9*, 81–93.

Miller, L. J. (2017). Creating a common terminology for play behavior to increase cross-disciplinary research. *Learning and Behavior*, *45*, 330–334.

Moberg, G. P. (1985). *Animal Stress*. Bethesda, MD: American Physiological Society.

Monaghan, P. (1997). Reports of 'virgin birth' of snakes prompt excitement and skepticism. *The Chronicles of Higher Education* (December 19, 1997). Retrieved from www.chronicle.com/article/reports-of-virgin-birth-of/101039.

Murphy, J. B. (2007). *Herpetological History of the Zoo and Aquarium World.* Malabar, FL: Krieger Publishing Co..

Murphy, J. B., Adler, K., & Collins, J. T. (Eds.) (1994). *Captive Management and Conservation of Amphibians and Reptiles. Contributions to Herpetology*. Ithaca, NY: Society for the Study of Amphibians and Reptiles.

Murphy, J. B., Ciofi, C., de La Panouse, C., & Walsh, T. (Eds.) (2002). *Komodo Dragons: Biology and Conservation*. Washington, DC: Smithsonian Institution.

Neaves, W. B. & Baumann, P. (2011). Unisexual reproduction among vertebrates. *Trends in Genetics*, *27*, 81–88.

Nichols, D. K. (2003). Tracking down the killer chytrid of amphibians. *Herpetological Review*, *34*, 101–104.

Norris, K. S. & Kavanau, J. L. (1966). The burrowing environment of the western shovel-nosed snake, *Chionactis occipitalis* Hallowell, and the undersand environment. *Copeia*, *1966*, 650–664.

Ohno, S. (1967). *Sex Chromosomes and Sex-linked Genes*. Berlin: Springer-Verlag.

Olena, A. (2017). Snake sex determination dogma overturned. *The Scientist*. Retrieved from www.the-scientist.com/?articles.view/articleNo/49814/title/snake-sex-determination-dogma-overturned.

Olsen, M. W. (1975). Avian parthenogenesis. *Agricultural Research Service, United States Department of Agriculture*, *65*, 1–82.

Olsen, W. W. & Marsden, S. J. (1954) Natural parthenogenesis in turkey eggs. *Science*, *120*, 545–546.

Pough, F. H. 1993. Zoo-academic research collaborations: How close are we? Herpetologica 49: 500-508.

Pruitt, J. N., Burghardt, G. M., & Riechert, S. E. (2012). Non-conceptive sexual behavior in spiders: A form of play associated with body condition, personality type, and male intrasexual selection. *Ethology*, *118*, 33–40.

Rabb, G. B. & Rabb, M. S. (1960). On the mating and egg-laying behavior of the Surinam toad, *Pipa pipa*. *Copeia*, *1960*, 271–276.

Radcliffe, C. W., Chiszar, D., & O'Connell, B. (1980). Effects of prey size on post-strike behavior in rattlesnakes (*Crotalus durissus*, *C. enyo*, and *C. viridis*). *Bulletin of the Psychonomic Society*, *16*, 449–450.

Schloegel, L. M., Toledo, L. F., Longcore, J. E., Greenspan, S. E., Vieira, C. A., Lee, M., . . . Davies, A. J. (2012). Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis associated with the bullfrog trade. *Molecular Ecology*, *21*, 5162–5177.

Schuett, G. W., Clark, R. W., Repp, R. A., Amarello, M., Smith, C. F., & Greene, H. W. (2016). Social behavior of rattlesnakes: a shifting paradigm. In G. W. Schuett, M. J. Feldner, C. F. Smith, & R. S. Reiserer (Eds.), *Rattlesnakes of Arizona, 2* (pp. 161–244). Rodeo, NM: ECO Publishing.

Schuett, G. W., Fernandez, P. J., Gergits, W. F., Casna, N. J., Chiszar, D., Smith, H. M., . . . Demlong, M. J. (1997). Production of offspring in the absence of males: Evidence for facultative parthenogenesis in bisexual snakes. *Herpetological Natural History*, *5*, 1–10.

Schuett, G. W., Fernandez, P. J., Chiszar, D., & Smith, H. M. (1998). Fatherless sons: A new type of parthenogenesis in snakes. *Fauna*, *1*, 20–25.

Schuett, G. W. & Booth, W. (2017). Facultative parthenogenesis in reptiles: Discovery and progress – Role of zoos and private citizens. Presented at: *International Herpetological Symposium*, Chiricahua Desert Museum & Geronimo Event Center, Rodeo, New Mexico, July 19–23.

Schuetz, A., Farmer, K., & Krueger, K. (2017). Social learning across species: Horses (*Equus caballus*) learn from humans by observation. *Animal Cognition*, *20*, 567–573.

Schut, E., Hemmings, N., & Birkhead, T. R. (2008). Parthenogenesis in a passerine bird, the zebra finch *Taeniopygia guttata*. *Ibis*, *150*, 197–199.

Serres, A. & Delfour, F. (2017). Environmental changes and anthropogenic factors modulate social play in captive bottlenose dolphins (*Tursiops truncatus*). *Zoo Biology*, *36*, 99–111.

Sharpe, S. S., Koehler, S. A., Kuckuk, R. M., Serrano, M., Vela, P. A., Mendelson, J. R., III, & Goldman, D. I. (2015). Locomotor advantages of being a slender and slick sand-swimmer. *Journal of Experimental Biology*, *218*, 440–450.

Tomaszkiewicz, M., P. Medvedev, and K. D. Makova1 (2017). Y and W chromosome assemblies: approaches and discoveries. Trends in Genetics 33: 266-282.

Vicoso, B., Emerson, J. J., Zektser, Y., Mahajan, S., & Bachtrog, D. (2013). Comparative sex chromosome genomics in snakes: Differentiation, evolutionary strata, and lack of global dosage compensation. *PLoS Biology*, *11*, e1001643.

Walker, M. (2011), Snake gives 'virgin birth' to extraordinary babies. Retrieved from news.bbc.co.uk/earth/hi/earth_news/newsid_9139000/9139971.stm.

Waters, R. M., Bowers, B. B., & Burghardt, G.M. (2017). Personality and individuality in reptile behavior. In J. Vonk, A. Weiss, & S. Kuczaj (Eds.), *Personality in Nonhuman Animals* (pp. 153–184). New York: Springer Publishing.

Watts, P. C., Buley, K. R., Sanderson, S., Boardman, W., Ciofi, C., & Gibson, R. (2006). Parthenogenesis in Komodo dragons. *Nature*, *444*, 1021–1022.

Weiss, R. (1997). Birds do it, bees do it, but some snakes may not need to. *The Washington Post*. Retrieved from www.washingtonpost.com/archive/politics/1997/09/15/birds-do-it-bees-do-it-but-some-snakes-may-not-need-to/caa108c6-4170-4229-991e-72b01f758411/?utm_term=.b1615d5d79fd.

Westlund, K. (2014). Training is enrichment – And beyond. *Applied Animal Behaviour Science*, *152*, 1–6.

Zylinski, S. (2015). Fun and play in invertebrates. *Current Biology*, *25*, R10–R12.