Rattlesnakes as Models for Reproductive Studies of Vertebrates

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With at least 15 species, Arizona boasts more kinds of rattlesnakes than any other state in the United States. This incredible diversity is not just of taxonomic significance, but also evolutionary. Adult body sizes, for example, range from diminutive, such as the Massasauga and Twin-spotted Rattlesnake, to very large, with the Western Diamond-backed Rattlesnake being the second largest species in the United States. This diversity also extends to the biomes and ecosystems they occupy, ranging from Sonoran Desert Scrub to Alpine Tundra, with tremendous variation in plant life, soil types, elevation, rainfall, temperature, density of human populations, and other factors. Some species, such as the Western Diamond-backed Rattlesnake and Black-tailed Rattlesnake, can be found in most of the biome types in Arizona. The diversity of rattlesnake species and the habitats they occupy in Arizona makes them excellent vertebrate models for many lines of biological inquiry. Chief among these is reproduction. Here, we review and synthesize what we currently know about the reproductive biology of rattlesnakes in Arizona and other southwestern states where they occur. Importantly, we identify and provide direction for key areas of future research.

Rattlesnake diversity and model organisms

To understand evolutionary causes and trends, investigating reproductive biology and life history (e.g., age and size at first reproduction, litter size) inherently requires studying animals in the field. The laboratory is a poor mimic of nature and hence largely unacceptable for the study of these variables (Krohmer et al., 1987; Taylor and DeNardo, 2005). Even mechanistic questions, such as how hormones regulate reproduction, are best studied in the field because it provides results that are ecologically relevant (reviewed by Taylor and DeNardo, 2010). However, in some studies, animals must be kept in the laboratory in order to isolate specific variables and or conditions (e.g., how food intake affects reproductive maturity; see Taylor and DeNardo, 2005). It is often difficult to find animal species that are amenable to investigating reproduction in the field, especially for extended lengths of time, while simultaneously being tractable to housing and reproduction in the laboratory.

Some species of rattlesnakes are especially suited to study in the field over extended periods of time (Beaupre and Duvall, 1998). There are at least five major reasons for this. First, some species are abundant where they occur (Klauber, 1972). Second, most rattlesnakes are ambush hunters, and tend to coil in ambush postures above ground regularly (Nowak et al., 2008; see Clark, this volume, Hunting and Feeding Behavior). This quality makes rattlesnakes less secretive than many other species of snakes. Because rattlesnakes can be abundant and visible, researchers can locate many subjects from which to collect data. Third, some rattlesnake species are relatively large-bodied, which permits certain techniques to be used that are not possible (or are limited) in species that are smaller. One important technique is radio-telemetry using surgically implanted radio-transmitters. Since the development of this technology in the 1980s, the size of transmitters has decreased and the length of battery life has increased, permitting the study of individual snakes for longer periods of time. Current technology permits the surgical implantation of a transmitter weighing 10–15 grams with a battery life of 2–3 years into an adult rattlesnake 1 m in length (see Beaupre, this volume, Monitoring Technologies). This is far superior to radio-telemetry that involves smaller individuals because battery life is severely limited in small radio-transmitters. Fourth, compared to many endothermic vertebrates, rattlesnakes have relatively small activity ranges (reviewed in Macartney et al., 1988), and thus locating them regularly with radio-telemetry is possible. With radio-telemetry, researchers can usually move on foot to locate snakes, whereas in the study of large birds and mammals, investigators commonly must use land vehicles, aircraft, or boats. The fifth reason that rattlesnakes make excellent study subjects for field studies is that they appear resilient to capture and manipulation. Some species do not appear to exhibit long-lasting behavioral and or physiological effects of being handled by researchers (Holding et al., 2014a). Together, these five attributes make rattlesnakes attractive organisms to study ecologically relevant questions in the field. Reproductive physiologists studying free-ranging rattlesnakes have many factors in their favor. If a field site is chosen carefully, then researchers can: a) study many snakes within a small area, b) implant long-lasting, economically priced radio-transmitters, c) have access to those snakes on a regular basis to collect blood samples because they tend to rest above ground, and d) be confident that their study techniques will have minimal negative effects on the snakes (Holding et al., 2014a). Indeed, it is challenging to identify other study organisms with this valuable combination of traits.

In addition to the abovementioned qualities that make certain rattlesnake species attractive models for field studies, most usually do well in captivity, which includes induction of reproductive behaviors (e.g., courtship, coitus) and production of offspring (Klauber, 1972; Murphy and Armstrong, 1978). Although adults collected from the wild can appear stressed in captivity and may not mate or produce offspring for several years despite access to food and water, after a period of acclimation many wild-caught adults eventually will reproduce (E. Taylor, unpubl. data). Laboratory-reared rattlesnakes, on the other hand, fare well in captivity and tend to reproduce readily. Recently, captive rattlesnakes have yielded interesting findings and discoveries, including long-term sperm storage by females and asexual reproduction via facultative parthenogenesis (Schuett, 1992; Schuett et al., 1997, 2004; Booth and Schuett, 2011, 2016).

Based on these attributes, we suggest that rattlesnakes are excellent model vertebrates for studies of reproduction both in the field and laboratory. Since the best studies represent a fusion of field-based observation, lab-controlled experimentation, and data derived from museum specimens (**Box 1**), it is clear that the benefits of studying rattlesnakes outweigh the potential costs associated with them being highly venomous. In our own work, very careful and minimal handling of study subjects greatly reduces the risk of human envenomation while also reducing any stress to the animals (see Beaupre and Greene, 2012).

Male reproductive cycle

The timing of spermatogenesis has been detailed in many species of snakes including rattlesnakes (reviewed in Taylor and DeNardo, 2010; DeNardo and Taylor, 2011). The species that occur in Arizona and other regions of the southwest in which spermatogenesis has been described include the Desert Massasauga, *Sistrurus tergeminus* (Goldberg and Holycross, 1999); Tiger Rattlesnake, *Crotalus tigris* (Goldberg, 1999b); Black-tailed Rattlesnake, *Crotalus molossus* (Goldberg, 1999a); Mohave Rattlesnake, *Crotalus scutulatus* (Goldberg and Rosen, 2000; Schuett et al., 2002); Twin-spotted Rattlesnake, *Crotalus pricei* (Goldberg, 2000); Ridgenose Rattlesnake, *Crotalus willardi* (Holycross and Goldberg, 2001); Arizona Black Rattlesnake, *Crotalus cerberus* (Goldberg, 2002); Western Diamond-backed Rattlesnake, *Crotalus atrox* (Goldberg, 2007); and Great Basin Rattlesnake, *Crotalus lutosus* (Glaudas et al., 2009). These papers show that all Arizona rattlesnakes show the aestival pattern of spermatogenesis, which is typical of all other species of North American pitvipers (for details, see Saint Girons, 1982; Schuett, 1992; Schuett et al., 2002; Graham et al., 2008). Sperm formation occurs in the summer, and these spermatozoa are stored in the vasa deferentia until the following spring and or summer mating season(s) when mating occurs. The various

Box 1. The use of museum specimens for investigating reproduction

Studies on live rattlesnakes (free-ranging and captive) have contributed greatly to our understanding of rattlesnake reproductive biology. However, preserved specimens represent an additional, extremely valuable resource for biological studies (Rocha et al., 2014), including reproductive biology (Schuett et al., 2002). The thousands of rattlesnakes collected from the wild, preserved, and deposited in natural history museums, represent a valuable resource for studies of reproduction, especially seasonal cycles. Much of our knowledge about various life history parameters comes from preserved specimens in collections. Specifically, the following traits have been studied in large part in preserved rattlesnake specimens (for studies conducted on Arizona rattlesnakes, see Table 1): timing of events in the reproductive cycle, frequency of reproduction in females, number and size of offspring, and size at reproductive maturity.

Why study reproduction in preserved specimens? Indeed, timing of mating can be observed directly, via observation of free-ranging snakes. However, even in studies involving daily radio-telemetry, some mating behaviors may be missed. Also, studying mating behavior only provides data about the timing of mating; it does not provide data on the timing of physiological events like spermatogenesis, vitellogenesis, ovulation, etc. Timing of vitellogenesis, ovulation, and the progression of gestation can be observed in free-ranging rattlesnakes via ultrasonography (Taylor et al., 2004). However, the vast majority of rattlesnake populations are not being studied by radio-telemetry, few studies track females at all, and fewer still have access to portable ultrasound equipment to bring to the field. Furthermore, certain events, such as spermatogenesis, can only be studied by examining testis tissue, and preserved specimens are obviously ideal for that purpose. Specimens have been collected and deposited in natural history museums over many decades, making it possible to study changes in reproduction over time through the use of museum specimens. The best studies therefore use both field and laboratory techniques, including the study of preserved specimens, to try to fully understand a species' reproductive biology.

As a word of caution, however, one must be confident in the certainty of species delineation of the museum specimen in order to avoid the introduction of noise that may reduce the accuracy of species-based studies (Bryson et al., 2014). Substantial problems can also occur when snakes are deposited without information about the time of death. It has become more commonplace to keep certain species alive, sometimes for months or years, before they are deposited. In these particular cases, reproductive information has been compromised and is of no value in studies of seasonal variables.

species show minor variation in the timing of recrudescence and regression, but all show the same basic seasonal pattern, indicating that the timing of spermatogenesis is largely conserved among Arizona rattlesnakes.

Female reproductive cycle

Preserved specimens of female rattlesnakes have proven equally informative in providing data on reproduction. Field studies of female rattlesnakes are still relatively rare, partly because females are usually smaller than males, and the burden of carrying a radio-transmitter might be higher for them, and possibly also because male rattlesnakes are easier to find in some populations than females. While radiotelemetry with portable ultrasonography studies provide excellent data on the timing of reproductive events (Taylor et al., 2004), these studies cannot reasonably be conducted on a scale suitable for studying the diversity of species and populations within Arizona. Historically and currently, preserved specimens of female rattlesnakes have provided important data on reproduction. Upon dissecting a female rattlesnake, researchers can measure and quantify the developing eggs and embryos, and in this way estimate the timing of vitellogenesis (production of the protein vitellogenin, which stimulates yolk deposition), ovulation (movement of the follicles from the ovaries to the oviducts, where fertilization occurs), and parturition (which occurs shortly after yolk depletion). In addition, researchers can quantify maximum litter sizes in this way (litter size quantified by counting enlarged, yolked follicles should be counted as potential maximum litter size because it is possible that the female could resorb one or more follicles, or that some fetuses would be underdeveloped or stillborn). Much of our current information on these life history parameters has been gleaned from preserved museum specimens rather than live snakes in the field (Table 1).

Arizona's high diversity of species and habitats translates into an array of female reproductive strategies. As stated above, most of our knowledge of these strategies comes from studies of preserved specimens, along with several field studies that have strengthened the data available on female rattlesnake reproduction (Prival et al., 2002; Taylor et al., 2004; Glaudas et al., 2009; Schuett et al., 2011, 2013a, b; Prival and Schroff, 2012). Without exception, female rattlesnakes give birth in the summer-fall months (July through early October). Summer parturition may be beneficial to offspring and or females for a number of reasons. First, offspring born in summer may have several months to hunt and improve body condition before overwintering, reducing the chances that they will starve before attaining a meal the following year. In many geographic areas, rodent and lizard abundances are high during the summer, providing ready food sources for neonate snakes. Second, summer parturition may be related to female body condition. By giving birth midway through the active season, females are able to put on enough weight in the spring to support their reproductive output, and then still have time to hunt and regain energy reserves before overwintering. Third, in many geographic areas, including Arizona, summer monsoons bring rainfall that may provide important water resources to pregnant female snakes and to their neonates (Schuett et al., 2011).

Although female rattlesnakes give birth in the summer and fall, they usually do not give birth every year. Whereas males typically at-

Table 1

Reproductive characteristics of rattlesnake species that occur in Arizona and the southwestern United States and Mexico. Within-row letters refer to the references cited at the end of each row.

Scientific Name	Common Name	Mating Season(s)	Frequency of Reproduction (Females)	Mean (+ Range) Litter Size	Smallest Mature Male (SVL, cm)	Smallest Mature Female (SVL, cm)	References
Crotalus abyssus	Grand Canyon	Summer	--	--	--	--	Reed and Douglas (2002)
C. atrox	Western Diamond-backed	Spring, Summer[a,c,f,g]	Every 2+ years[b,d,f,h], Sometimes annual[d,h]	7.3 (4-15)[b] 3.4 (1-9)[e] 4.5 (2-7)[f]	54.1[a]	64.8[b]	Goldberg (2007)[a] Rosen and Goldberg (2002)[b] Schuett et al. (2005)[c] Schuett et al. (2011)[e] Schuett et al. (2012)[d] Taylor and DeNardo (2005)[f] Taylor et al. (2004)[g] Taylor et al. (2005)[h]
C. cerberus	Arizona Black	Summer[a]	Every 2+ years	5.8 (2-9)[a] 10[b]	56.2[a]	53.9[a]	Goldberg (2002)[a] Sievert (2002)[b]
C. lutosus	Great Basin	Summer	Every 2+ years	--	50.8	51.4	Glaudas et al. (2009)
C. molossus	Black-tailed	Summer [a,b]	Every 2+ years	5.2 (2-13)[a]	57.6[a]	65.3[a]	Goldberg (1999a)[a] Schuett et al. (2005)[b]
C. pricei	Twin-spotted	Summer[b,c]	Every 2+ years[a-c], Sometimes annual [c]	5.1 (3-8)[a] 3.9 (1-6)[b] 4.3 (1-6)[c]	32.2[a]	33[a]	Goldberg (2000)[a] Prival et al. (2002)[b] Prival (2012)[c]
C. scutulatus	Mohave	Spring, Summer[a,b]	Every 2+ years[a]	8.2 (5-13)[a]	41.1[a]	61.1[a]	Goldberg and Rosen (2000)[a] Schuett et al. (2002)[b]

Table 1 Continued

C. tigris	Tiger	Summer	Every 2+ years, Sometimes annual	4.2 (3-6)	51.2	54.1	Goldberg (1999b) Goode et al. (volume 1, *Crotalus tigris*)
C. willardi	Ridgenose	Summer[a]	Every 2+ Years[a]	5.4 (2-9)[a] 6[b]	40.6[a]	40.2[a]	Holycross and Goldberg (2001)[a] Martin (1975)[b]
Sistrurus tergeminus	Massasauga	Spring (?), Summer	Every 2+ Years	5.8 (4-8)	28	32.9	Goldberg and Holycross (1999)

tempt to mate annually, female rattlesnakes tend to reproduce at a frequency of once every two or more years (Table 1). The predominant hypothesis explaining less than annual reproduction in female rattlesnakes involves energy. Female snakes spend so much energy on reproduction, sometimes they lose up to 40% or more of their mass from parturition (Seigel et al., 1986), and it often takes them one or more years to regain sufficient energy reserves to reproduce again. Most female rattlesnakes initiate vitellogenesis in the fall, continue it the following spring, ovulate in the late spring, and gestate during the summer. Fertilization occurs at the time of ovulation, and uses sperm procured during the concurrent mating season and or stored in the oviducts from previous matings (see below). In these cases, it is difficult to imagine how an emaciated, post-parturient female could possibly hunt enough in the 2–3 months following parturition to gain sufficient energy to begin vitellogenesis before winter. Interestingly, in some populations *C. atrox* do not begin vitellogenesis in the fall, but rather initiate it rapidly during the following spring (Schuett et al., 2004; Taylor et al., 2004; see Schuett et al., 2011). Also, whereas most female rattlesnakes become anorexic during pregnancy, *C. atrox* females readily continue eating throughout pregnancy (Taylor et al., 2005; Schuett et al., 2013a). These factors may allow *C. atrox* females to attain high enough body condition to reproduce annually when food availability is high (Schuett et al., 2011). In support of this hypothesis, free-ranging female *C. atrox* that were supplementally fed during pregnancy were able to reproduce annually during a severe drought when other females did not reproduce (Taylor et al., 2005). In contrast to the highly fecund *C. atrox*, at the other end of the spectrum is the Great Basin Rattlesnake, *C. lutosus*. In Nevada, females initiate vitellogenesis in the spring, but they do not ovulate until the following spring (Glaudas et al., 2009). This means that the maximal reproduction rate is biennial or less frequently. In addition to frequency of reproduction, the average number and size of offspring in a given litter are important determinants of fecundity. These variables depend on species and population (Table 1), and likely on various environmental factors like rainfall, food availability, temperature, length of the active season, and other factors (see Schuett et al., 2011).

Hormonal regulation of reproduction

The relationships between hormones and events in the reproductive cycle have been investigated in both male and female rattlesnakes, including those in Arizona (Schuett et al., 2002, 2004, 2005, 2006; Taylor et al., 2004). The ability to study free-ranging rattlesnakes has facilitated a fairly detailed understanding of how steroid hormones may be related to reproductive events in various species (reviewed in Taylor and DeNardo, 2010; DeNardo and Taylor, 2011). Typically, free-ranging snakes are captured, restrained, and a blood sample is drawn from the caudal blood vessels as soon as possible to prevent the stress-induced changes in glucocorticoid hormones that occur after several minutes of handling in rattlesnakes (Holding et al., 2014a). Because rattlesnakes are large-bodied and tend to be found frequently above ground, scientists can collect a lot of blood from many snakes, permitting samples of sufficient size to analyze the potential roles of several different hormones over the course of the reproductive cycle. Although similar data exist for several colubrid species, and several species from other major lineages, the relationship between steroid hormone concentrations and reproduction in free-ranging snakes has been studied in no other snake taxonomic group as extensively as in rattlesnakes (Taylor and DeNardo, 2010; DeNardo and Taylor, 2011).

The major hormones that have been studied in research on rattlesnake reproduction are androgens (mainly testosterone, T, and to a lesser extent its metabolite dihydrotestosterone, DHT), estrogens (17β-estradiol, E2), progestogens (progesterone, P4), and glucocorticoids (corticosterone, CORT) (**Box 2**). Because E2 and P4 tend to be in high concentrations only in females, and because the majority of research on rattlesnakes is performed on males, most studies report only T and CORT hormone concentrations. The next sections describe the knowledge we have gained to date about the potential roles of steroid hormones in reproductive behavior and physiology in rattlesnakes.

Androgens and reproduction in male rattlesnakes

As in many other species, the androgen T tends to be elevated during spermatogenesis and mating in rattlesnakes (Schuett et al., 2002, 2005, 2006). Spermatogenesis, an androgen-dependent process (Smith and Walker, 2014), broadly consists of the recrudescence of testicular and associated tissues (epididymides, vasa deferentia, sexual segments of the kidneys) along with early cell division of spermatogonia, followed by spermiogenesis, or sperm formation, where mature sperm enter the epididymides and are stored in the vasa deferentia until ejaculation. In all species of rattlesnake studied so far, males have elevated concentrations of plasma androgens during the latter stages of spermatogenesis, which occurs in late summer (reviewed in Taylor and DeNardo, 2010; DeNardo and Taylor, 2011).

The sexual segment of the kidney is often hypertrophied during spermatogenesis due to the influence of androgens (see Schuett et al., 2002). Although its function is largely unknown, it has been hypothesized to function in one or more ways related to mating, including sperm activation and or contribution/s to semen (Sever et al., 2012). In those species with a single summer mating season, it is not possible to conclude that mating behaviors like mate-searching, courtship, copulation, and mate-guarding are androgen-dependent, because they could instead be elevated to stimulate spermatogenesis. Indeed, although androgens tend to stimulate mating behavior in most vertebrate species, mating in some species may not require elevated levels of circulating T. For example, Timber Rattlesnakes (*Crotalus horridus*) in Arkansas mate in the late summer months when androgen levels have already dropped back to or near baseline following an early summer peak (Lind and Beaupre, 2014).

An oft-cited example of this “dissociated” reproductive cycle is the Red-sided Garter Snake (*Thamnophis sirtalis parietalis*), whose reproductive biology has been studied extensively (reviewed in Taylor and DeNardo, 2010; DeNardo and Taylor, 2011). Male Garter Snakes court and copulate with females eagerly even when their plasma T levels are extremely low (Camazine et al., 1980). The distinction between this “dissociated” cycle and those in which mating behaviors and elevated hormone levels are “associated” has become blurred even in the Garter Snake, however, with the finding that free-ranging male Garter Snakes have elevated T levels during both their spring and summer mating seasons (see Krohmer et al., 1987; Moore et al., 2000).

Comparing the androgen concentrations of rattlesnake species with different mating seasons has proven instrumental in demonstrating the role of androgens in mating behavior (Schuett et al., 2002, 2005; Taylor et al., 2004; see Graham et al., 2008). Although spermatogenesis and mating occur at the same time in species with a single summer mating season, and are therefore confounded with respect to the role of T, many species of rattlesnakes exhibit an additional spring mating season that is dissociated from the androgen-dependent stages of spermatogenesis. If T stimulates mating behavior, then T concentrations should be elevated in the spring even when peak spermatogenesis is not occurring, whereas they should be low in the spring in those species that do not mate in the spring. These predictions have been upheld so far in studies conducted on Arizona rattlesnake species with spring and late summer mating seasons, which exhibit bimodal peaks in T associated with these mating seasons (*C. atrox*, Taylor et al., 2004; Schuett et al., 2005, 2006; *C. scutulatus*, Schuett et al., 2002), whereas the T levels are low in the spring in species that mate only in the late summer (*C. molossus*, Schuett et al., 2005). These data provide strong circumstantial evidence in male rattlesnakes and other pitvipers that androgens stimulate reproductive behaviors important to locating and successfully courting and copulating with females (Graham et al., 2008; Lind and Beaupre, 2014).

Hormones and reproduction in female rattlesnakes

Much less is known about the hormonal regulation of reproductive behavior in wild female rattlesnakes, but field studies using portable ultrasonography and blood sampling for hormone quantification have provided basic information about the roles of hormones in fe-

Box 2. Corticosterone and reproduction in rattlesnakes

Corticosterone (CORT), the major glucocorticoid in reptiles, is not a major sex steroid hormone like T, E2, and P4, but it nonetheless may be important in reproduction. The main role of CORT is to mobilize energy when it is needed, such as in response to various types of stressors (Romero, 2002; Moore and Jessop, 2003). Reproduction certainly falls into this category: males may expend a lot of energy travelling long distances in search of females, and pregnant females can invest 40% of their body mass or more to produce a litter (Seigel et al., 1986). Thus, elevated CORT may be associated with energetically taxing reproductive events in rattlesnakes. However, CORT also increases rapidly in response to acute stressors, such as encounters with predators or capture by scientists (Holding et al., 2014a). Even in the absence of stress, CORT levels may be affected by numerous other factors such as temperature, time of day, recent meals, and others (Holding et al., 2014b). These issues make interpretation of the specific role of CORT in reproduction difficult. So far, only one major pattern has emerged with respect to the role of CORT in reproduction of rattlesnakes. CORT is elevated during gestation in female *C. atrox*, and drops immediately prior to parturition (Taylor et al., 2004) or during parturition (Schuett et al., 2004). This suggests that CORT either helps mobilize energy to fuel the increased metabolic demands of pregnancy, or that it plays another role in pregnancy and or parturition. Further studies on the role of CORT in reproduction in rattlesnakes and other pitvipers are clearly needed because CORT is such a multi-faceted, complicated hormone (see Hoss et al., 2014).

male reproductive physiology (reviewed in Taylor and DeNardo, 2010). Both E2 and T are elevated in female *C. atrox* during vitellogenesis (Schuett et al., 2004; Taylor et al., 2004). Estradiol stimulates vitellogenesis in female reptiles (Ho et al., 1982), leading to the mobilization of energy reserves to be converted to yolk. Testosterone levels are far lower in female rattlesnakes than in males, but are nonetheless elevated during vitellogenesis (Schuett et al., 2004; Taylor et al., 2004). Since T is a precursor to E2 in the steroid hormone biosynthetic pathway, it is possible that the small elevation in circulating T concentrations in vitellogenic females is simply residual T produced while making E2. Alternatively, androgens are known to stimulate receptivity in females of some other reptile species (reviewed in Staub and De Beer, 1997), so it is possible that elevated T plays a role in mating behavior. Because most female rattlesnakes begin vitellogenesis in the late summer and continue it in the spring, corresponding to both potential mating seasons, it is difficult to unravel the role of androgens in female reproduction in rattlesnakes. However, unlike T and other steroids, the functional role of P4 is clearer. Just as in all viviparous female vertebrates, P4 is elevated during gestation in rattlesnakes (Schuett et al., 2004; Taylor et al., 2004), as it plays a role in maintaining the placenta and other fetal-maternal tissues during pregnancy (Mead et al., 1981; reviewed in Custodia-Lora and Callard, 2002). In summary, elevated E2 in plasma samples from females is a reliable indicator of vitellogenesis, and elevated P4 is a reliable indicator of pregnancy. Similarly, low levels of these hormones during the appropriate times of year are reliable indicators that a given female is not reproductive.

Reproductive strategies in rattlesnakes

Alternative mating strategies are important in determining patterns of gene flow and the distribution of genetic diversity (Petrie and Kempenaers, 1998; Avise, 2004; Rafajlović et al., 2013). While the majority of studies on vertebrates have focused on mammals, birds, and fishes, they reveal how the consequence of variation in the frequency with which different strategies are utilized may be far reaching, ultimately influencing the evolution of mating systems, dispersal, effective population size, social structure, and reproductive success (Emlen and Oring, 1977; Greenwood, 1980; Shields, 1987; Duvall et al., 1992, 1993; Nunney, 1993; Sugg and Chesser, 1994; Møller and Cuervo, 2000). The ability to address such variation, however, has long been hampered by the difficulty in assigning parentage in species with cryptic breeding habits (Prosser et al., 2002; Clark et al., 2014). However, in recent years the study of mating systems and alternative reproductive strategies has become revolutionized by the relative ease with which molecular markers may be developed and applied (e.g., Castoe et al., 2010, 2012; Booth et al., 2014; Clark et al., 2014), thus permitting individual identification and the accurate assignment of parentage (Gibbs and Weatherhead, 2001; Avise, 2004; Uller and Ollsson, 2008; Levine et al., 2015).

As such, it is now possible to tease apart associations that are purely social from those with actual genetic relatedness (Gibbs and Weatherhead, 2001; Clark et al., 2012, 2014; Pozarowski et al., 2013), an aspect previously beyond the scope of behavioral based research. Despite its application to a variety of squamate reptiles (Uller and Olsson, 2008), and specifically snakes (Prosser et al., 2002; Weatherhead et al., 2002; Blouin-Demers et al., 2005; Dubey et al., 2009; Ursenbacher et al., 2009; Booth et al., 2011a, b; Reynolds et al., 2012), relatively few species of crotalines (pitvipers) have been exposed to molecular analyses of breeding systems (Booth and Schuett, 2011; Siminov and Wink, 2011; Booth et al., 2012; Levine, 2013; Pozarowski et al., 2013; Clark et al., 2014; Levine et al., 2015, 2016; Lind et al., 2016). Our understanding of the diversity of reproductive strategies within this group is, therefore, limited. However, the reproductive biology of North America's pitvipers investigated from a behavioral perspective has received considerable attention (Gibbons, 1972; Diller and Wallace, 1984; Macartney and Gregory, 1988; Brown, 1991; Graves and Duvall, 1993; Martin, 1993; Beaupre, 1995; Goldberg, 1999a, b, 2000; Ashton et al., 2001; Holycross and Goldberg, 2001; Taylor and DeNardo, 2005; Cardwell, 2008; Schuett et al., 2013a, b; Hoss et al., 2015); therefore, it is likely to be just a matter of time before genetic parentage analyses are performed across more species.

To date, the genus *Thamnophis* has stood out as being the proverbial model system for studies of reproduction and mating behaviors in snakes (Tinkle, 1957; Devine, 1977; Garstka et al., 1982; Ford and Karges, 1987; Krohmer et al., 1987, 2004; Gregory and Larsen, 1993; Moore et al., 2000; Shine et al., 2000; Sparkman et al., 2007; Luttershmidt, 2009; Luttershmidt and Mason, 2009; Castoe et al., 2011; Reynolds et al., 2012; Friesen et al., 2013, 2014a, b). Widespread over much of North America, this speciose genus exhibits many characteristics common with temperate pitvipers. For example, many undergo seasonal hibernation events, denning communally, and, in some cases, mating upon leaving the hibernaculum in the spring (e.g., Carpenter, 1952; Gregory, 1977; Bronikowski and Arnold, 1999; Lutterschmidt et al., 2006). Litters may be sired by multiple males (Schwartz et al., 1989; McCracken et al., 1999; Garner et al., 2002; Garner and Larsen, 2005), long-term sperm storage may be prevalent (Friesen et al., 2013, 2014a), and facultative parthenogenesis has been recently documented in several species (Schuett et al., 1997; Germano and Smith, 2010; Reynolds et al., 2012). To some degree, members of *Thamnophis* exhibit behaviors and physiological events that have also been documented in North American pitvipers (Clark et al., 2008, 2011, 2014; Booth and Schuett, 2011; Gienger and Beck, 2011; Amarello, 2012; Fitzgerald et al., 2013). As such, given the commonalities exhibited between *Thamnophis* and pitvipers, it is clear that both groups represent ideal model systems to study reproductive biology.

Mating systems

Classically, mating systems were categorized according to the number of mates a male or female may be associated with based upon social observations (see Duvall et al., 1992, 1993; Arnold and Duvall, 1994; Shuster and Wade, 2003). Owing to the cryptic nature of most snake species and lack of parental care (Shine, 1988; but see Greene et al., 2002; Amarello, 2012; Clark et al., 2012; see Hoss et al., 2015 for a review of parental care in snakes), the potential to observe copulation, gestation, and parturition in the field across large numbers of individuals is limited (but see Schuett et al., 2011, 2013b). As evidenced by the significant body of research on avian species (Griffith et al., 2002), following the application of molecular tools it is clear that social mating systems are rarely tantamount to genetic mating systems (for discussions, see Booth et al., 2011a; Clark et al., 2014). Aside from the accurate discrimination of paternity (Prosser et al., 2002; Clark et al., 2014; Levine et al., 2015; Lind et al., 2016), the application of molecular tools permits an in-depth understanding of the genetic relationships between mating individuals, i.e., whether mating is random, positively assortative (inbreeding), or negatively assortative (outbreeding) (Shuster and Wade, 2003). In concert with an accurate understanding of mating system, such data may prove particularly informative in the investigation of sexual selection and competition within natural populations (Weatherhead et al., 2002; Kissner et al., 2005; Levine et al., 2015). Despite the glaring inconsistencies often observed between social and genetic mating systems (see Smith and Schuett, 2015; Smith et al., 2015), and the significant power of molecular tools, relatively few studies have focused on their application to infer mating system in snakes (e.g., Höggren and Tegelström, 1995; Weatherhead et al., 2002; Kissner et al., 2005; Madsen et al., 2005; Friesen et al., 2013; Levine, 2013; Clark et al., 2014; Levine et al., 2015, 2016; Lind et al., 2016).

Despite the relative paucity of studies documenting the genetic mating system in rattlesnakes, behavioral and physiological observations prove informative when viewed alongside those recently published incorporating molecular tools. Indeed, when doing so, a number of factors become evident that suggest monogamy may be uncommon, and instead multiple paternity and promiscuity are likely a common occurrence across rattlesnakes and other pitvipers. Specifically, we contend that: 1) many viperid snakes, including rattlesnakes, are long-lived (> 20 yrs) (Klauber, 1972; Brown, 1991; Prival and Schroff, 2012; Clark et al., 2014) and thus reproductive events are likely to occur multiple times over their lifetime. As such, even if a female produced a litter solely sired by a single male, the likelihood that the same male would sire all of her offspring in subsequent reproductive events is unlikely; 2) the operational sex-ratio (i.e., the number of reproductive males to reproductive females) is often likely to be skewed due to the non-annual female reproductive cycle (Table 1). Given that attendance and courtship among rattlesnake pairs can be protracted, often spanning days or weeks before mating is effected (Duvall et al., 1992, 1993; Duvall and Schuett, 1997), and the mating season can be relatively short, it is unlikely that a given male will devote resources for reproduction to a female in a year in which she is not reproductively receptive. Thus with more reproductively active males within a given population than females, and given that courtship and coitus do not guarantee paternity (Clark et al., 2014), females are unlikely to focus reproductive efforts solely on a single male; 3) sperm-storage has been documented in both males (i.e., the production of sperm prior to hibernation) and females (i.e., sperm stored from copulations the previous season that is utilized the following year) (see below). If multiple males copulate with a female prior to sperm storage, or upon emergence the following spring, ample opportunities exist for sperm mixing, and thus multiple paternity within single litters; and 4) despite small litter sizes often reported for Arizona rattlesnakes (Table 1), multiple paternity has been found to be common in at least one species exposed to molecular screening.

Clark et al. (2014) documented reproductive success and sexual selection in a single population of *C. atrox* in south-central Arizona across a period of 10 consecutive years. Multiple paternity was detected in 12 of 24 litters suitable for multiple paternity identification (i.e., three or more offspring, permitting the identification of greater than two paternal alleles, or two or more offspring assuming all or most males in the study area are genotyped and thus offspring mapped back to parents). In five of these cases involving multiple paternity, the litters were sired by three males, and in two cases the litter size was three. Furthermore, in multiple instances it was found that the males found to be copulating with a female sired no offspring, emphasizing the need for molecular identification of parentage. Examples of *Crotalus horridus* sampled in Arkansas are reported as having small litter sizes, yet in three of seven litters screened with molecular markers multiple paternity was detected (Lind et al., 2016). It is, therefore, evident from these studies that while litter size may be small for many species of rattlesnakes (Table 1), this does not relegate those species to genetic monogamy within single reproductive events; molecular identification of paternity within litters is thus essential (see Levine et al., 2015).

Long-term sperm storage

Among ectothermic organisms, the asynchronous nature of the timing of spermatogenesis, mating, and ovulation may present prolonged periods before sperm may be utilized (Birkhead and Møller, 1993). In many species that undergo winter hibernation, mating may occur prior to entering hibernation, with ovulation following emergence in the spring (Schuett, 1992; Smith et al., 2009); however, in those that do not utilize hibernation (e.g., tropical species) the presence of sperm storage over prolonged periods may exist to promote post-copulatory prezygotic competition among the sperm of different males (Birkhead, 2000; Schuster and Wade, 2003). Through delaying fertilization, a mechanism is therefore offered by which a female may maximize her fitness benefits (Fox, 1963; Sever and Brizzi, 1998). Observational records of sperm storage in snakes range from a few months to multiple years (reviewed in Saint Girons, 1975; Devine, 1984; Gist and Jones, 1989; Schuett, 1992; Olsson and Madsen, 1998; Sever and Hamlett, 2002; Holt and Loyd, 2010). However, only in several species of turtle (Pearse and Avise, 2001; Pearse et al., 2001; Roques et al., 2004, 2006; Johnston et al., 2006) and one species of snake have cases of long-term sperm storage (LTSS) been genetically confirmed, thus disentangling the potential for offspring to have resulted from facultative parthenogenesis (see Booth and Schuett, 2011). Indeed, it is a record from a *Crotalus adamanteus* that presents the longest genetically confirmed documentation of LTSS in any vertebrate species, totaling 67 months and resulting in 19 viable offspring and zero developmental failures (stillborns, deformed, or infertile ova) (Booth and Schuett, 2011). While the capacity for sperm storage has been documented in a variety of snake species (Haines, 1940; Halpert et al., 1982; Aldridge, 1992; Davenport, 1995; Sever and Ryan, 1999), it appears widespread in pitvipers (Ludwig and Rahn, 1943; Schuett and Gillingham, 1986; Schuett, 1992; Almeida-Santos and Salomão, 1997; Almeida-Santos et al., 2004; Schuett et al., 2004, 2005; Siegel and Sever, 2006; Smith et al., 2009, 2010, 2012; Booth and Schuett, 2011). Accordingly, a wealth of information is available proposing the mechanisms by which sperm may be stored, although within pitvipers (crotalines) some appear contradictory (e.g., Almeida-Santos et al., 2004; Siegel and Sever, 2006).

The oviducts of snakes can be divided into three defined regions: the infundibulum (positioned anteriorly), the uterus (middle), and the vagina (posterior). Each may then be divided into anterior and posterior regions (Blackburn, 1998). Within the Typhlopidae and Colubridae, sperm is believed to be stored in the posterior region of the infundibulum within sperm-storage tubules (SST) (Fox and Dessauer, 1962); however, temporary storage in the vagina and posterior region of the uterus may also occur (Saint-Girons, 1975; Halpert et al., 1982; Perkins and Palmer, 1996). In crotalines, however, histological studies suggest sperm may be stored in the anterior region of the vagina and the posterior region of the uterus (Ludwig and Rahn, 1943; Almeida-Santos and Salomão, 1997), through the process of uterine muscular twisting (UMT), a recently proposed mechanism by which the region becomes convoluted and contracted, sequestering the sperm in the oviduct until ovulation (Almeida-Santos and Salomão, 1997; Siegel and Sever, 2006). While investigated in the Neotropical Rattlesnake *Crotalus durissus*, the authors also suggest that UMT may be present in several species of *Agkistrodon* and in *Crotalus viridis* (Almeida-Santos and Salomão, 1997). Siegel and Sever (2006), however, failed to find evidence for UMT during histological examination of multiple specimens of *A. piscivorus* and in single specimens of *C. durissus* and *Sistrurus miliarius*. Instead, the authors found support for infundibular sperm storage (Siegel and Sever, 2006, 2008), with histochemical and ultrastructure analyses suggesting that sperm remaining in the posterior uterus degrade prior to ovulation. The detection of sperm within these posterior regions may therefore provide no indication of the actual function (or lack of) of these regions in LTSS.

In a recent study macroscopically examining 76 preserved specimens of *C. durissus*, Barros et al. (2012) found evidence supporting UMT in the posterior uterus of 'some' females collected during autumn and winter, but not in those collected during the spring or summer. Actual numbers exhibiting UMT were, however, not presented. The authors suggest sperm may remain viable due to interactions with secretions produced by either the male or the female, as previously proposed by both Siegel and Sever (2008) and Marinho et al. (2009). The presence of structures that appear to be STT in the posterior infundibulum was reported, however no sperm was found in these, suggesting they may play a role in storing sperm prior to ovulation (Siegel and Sever, 2006). It is clear that the mechanisms for and anatomical structures used in LTSS are still up for debate. The subject of LTSS may represent a rich area for future research, given the potential application to human and livestock sperm storage under non-cryogenic conditions. As a result of the variation observed in the Crotalinae, we therefore propose this group as an ideal model for future studies of sperm storage in snakes.

Facultative parthenogenesis

Within vertebrates, obligate parthenogenesis is a reproductive trait restricted, based upon current understanding, to the squamate reptiles (Avise, 2008). While documented across a variety of lizard taxa (Crews et al., 1986; Dawley and Bogart, 1989; Murphy et al., 2000; Stasburg and Kearney, 2005; Sinclair et al., 2010), it is known in only a single species of snake, the Brahminy Blind Snake (*Indotyphlops braminus*). This geographically widespread basal scolecophidian exists as all female populations, is believed to be triploid, and in common with many obligately parthenogenetic lizards has been hypothesized to have resulted from hybrid origin (Nussbaum, 1980; Wynn et al., 1987; Ota et al., 1991). In contrast, facultative parthenogenesis (FP) — asexual reproduction by otherwise sexually reproducing species — has been documented in a variety of snakes and appears to be phylogenetically widespread (Dubach et al., 1997; Schuett et al., 1997; Groot et al., 2003; Germano and Smith, 2010; Booth and Schuett, 2011, 2016; Booth et al., 2011a, b, 2012, 2014; Kinney et al., 2012; Reynolds et al., 2012; W. Booth and G. Schuett, unpubl. data).

Given this plethora of recent studies, it is evident that the cytogenic mechanism of automixis has been conserved across lineages (Booth and Schuett, 2016); but see Portnoy et al. (2014) for an alternative mechanism recently discovered in vertebrates. Without confirmation of the presence or absence of heterozygosity, however, the actual mode of development—either terminal fusion or gametic duplication—cannot yet be conclusively assigned (Booth and Schuett, 2016). Regardless, under these automictic modes, the offspring produced are half-clones of the mother, resulting from the fusion of a haploid egg with the second polar body produced during meiosis II (terminal fusion), or the division of a haploid egg to produce cleaved nuclei that subsequently fuse (gametic duplication), which thus restores the diploid state and ultimately mimics fertilization (Lampert, 2008; Booth and Schuett, 2016). To date, all species of caenophidian (advanced) snakes have been assigned to a ZZ/ZW system of sex determination, with females being the heterogametic (ZW) sex (Matsubara et al., 2006; Vicoso et al., 2013). Through automictic FP, the resulting offspring may, therefore, be either male (ZZ), or female and presumed to be WW (Figure 1) — the latter is a chromosomal arrangement previously considered as non-viable until recent work on basal alethinophidians of multiple, viable, female Boa Constrictor (*Boa constrictor*, *B. imperator*), Brazilian Rainbow Boa (*Epicrates cenchria*), Colombian Rainbow Boa (*Epicrates maurus*), Reticulated Python (*Malayopython reticulatus*), Royal Python (*Python regius*) (Booth et al., 2011a, b, 2014; Kinney et al., 2013), and Green Anaconda (*Eunectes murinus*) (O'Shea et al., 2016). However, in contrast to a WW state, Booth and Schuett (2016) recently formulated an alternative hypothesis referred to as the Directionality of Heterogamety, under which both boids and pythonids (perhaps other basal taxa) may possess an XX/XY sex-determining system with female homogamety (XX). This hypothesis most parsimoniously explains the production of only female parthenogenetic offspring (Figure 1). To date, the only evidence supporting the production of clonal females through mechanisms other than terminal fusion automixis or gametic duplication has come from pythons (Groot et al., 2003). Nonetheless, a recent publication by Booth et al. (2014) has

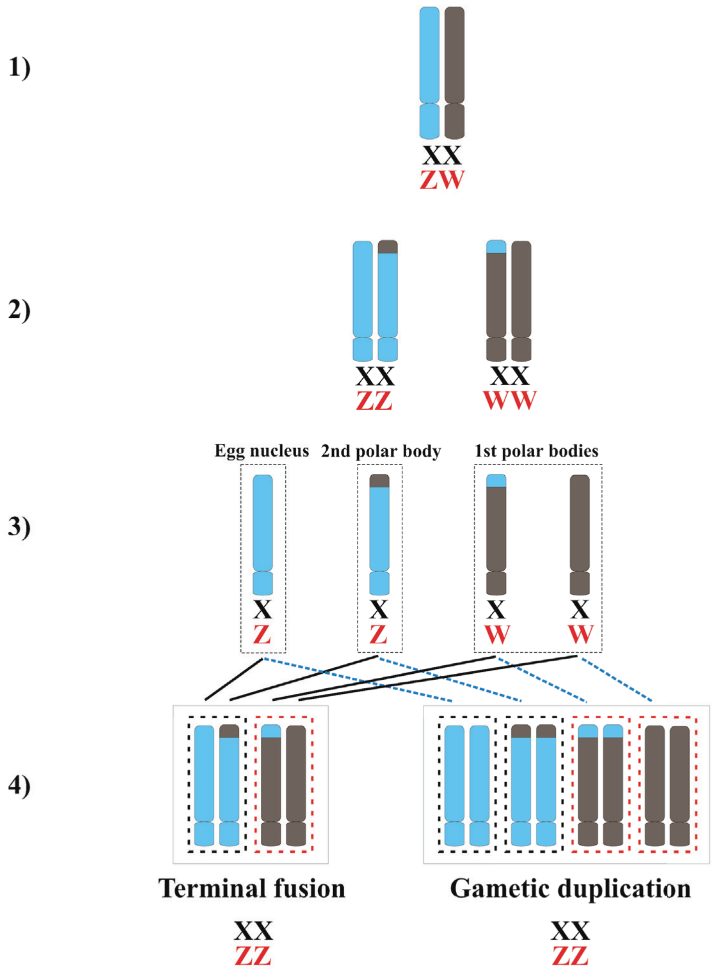

Figure 1. Mechanisms of automixis in snakes. Black = Type A (basal alethinophidians), Red = Type B (caenophidians) (Booth and Schuett, 2016). 1) Primordial germ cell. 2) Meiotic products following DNA replication and recombination during the first cell division. 3) Meiotic products following second cell division. 4) Potential sex chromosomal arrangements following terminal fusion (black lines) and gametic duplication (blue lines). Note that WW arrangements (depicted in red dashed-lined boxes) are considered non-viable under Type B parthenogenesis (Booth and Schuett, 2016).

questioned the validity of this report; instead, these authors provide evidence supporting terminal fusion automixis or gametic duplication, and, therefore, the generation of offspring that are essentially half-clones.

Through phylogenetic analyses of the characteristics associated with FP in snakes, it has become evident that two distinct forms exist (Booth and Schuett, 2016). Within the basal alethinophidians, FP (referred to as Type A) is characterized by moderate to large litters/clutches, consisting only of females and previously assumed to be WW, but the true state may be XX. In contrast, in caenophidian taxa showing FP (referred to as Type B, we see a contrasting pattern of small litters often comprised of stillborn or deformed male (ZZ) offspring; also, a large numbers of developmental failures. This latter type of FP is documented thus far in *Acrochordus* (Dubach et al., 1997), New World natricines (e.g., *Nerodia*, *Thamnophis*) and pitvipers (e.g., *Agkistrodon* and *Crotalus*) (Schuett et al., 1997; Booth and Schuett, 2011, 2016; Booth et al., 2012; Reynolds et al., 2012; Jordan et al., 2015; Booth et al., unpubl. data). In pitvipers, FP has been documented in the Timber Rattlesnake (*C. horridus*), Aruba Island Rattlesnake (*Crotalus unicolor*) (Schuett et al., 1997), and Prairie Rattlesnake (*C. viridis*) (Booth and Schuett, 2016). In the abovementioned species, which comprises three litters, live offspring were produced in two (*C. horridus* and *C. viridis*). While the location or fate of the single live male offspring produced in *C. horridus* cannot be determined, it was reported as outwardly normal in appearance and showed subsequent normal growth (see Schuett et al., 1997). In the latter two cases, however, the offspring were born deformed and later died. Additionally, FP has been observed in the Eastern Diamond-backed Rattlesnake (*C. adamanteus*) and the Western Massasauga (*Sistrurus tergeminus*) (W. Booth et al., unpubl. data). Eight other instances of FP have been documented in New World pitvipers, specifically the Copperhead (*A. contortrix*), the Eastern Cottonmouth (*A. piscivorus*), and the Terciopelo (*Bothrops asper*) (Booth and Schuett, 2011, 2016; Booth et al., 2012; Vaughan and Steele, 2014; Jordan et al., 2015). Deformations of the hemipenes have not been recorded in these three species, and at least two healthy, parthenogenetic individuals exist at the time of writing (*A. contortrix* – The Copperhead Institute: website: http://www.copperheadinstitute.org; *S. tergeminus*, Arizona-Sonora Desert Museum).

With the documentation of FP in *Crotalus*, *Agkistrodon*, and *Bothrops*, and the availability of live parthenogens—and in at least one case the mother (Booth et al., 2012) — in captivity, pitvipers such as rattlesnakes and their relatives, represent ideal models for the study of FP in the advanced snakes. Specifically, given its identification in natural populations of two species of *Agkistrodon* (Booth et al., 2012), the dogma that it is a syndrome of captivity is overturned, and thus the ecological and evolutionary significance may be realized and, therefore, warrants robust investigation (Booth and Schuett, 2016). As such, it may be addressed from a number of angles. Given the existence of live parthenogenetic individuals, it may now be possible to address the question of reproductive competence. We may presume that under natural conditions parthenogenesis may represent an evolutionary mechanism utilized during periods of mate absence, e.g., following invasion of new habitats, under conditions of habitat fragmentation, and so on. Determining whether a male produced by FP may then successfully produce offspring with a female will allow us to then understand the evolutionary trade-offs between the limited reproductive benefit received (i.e., small litter size; developmental failure or stillborn) and the potentially detrimental effects of reproduction with a male that is homozygous over the majority of its genome.

Given the reduced viability observed across parthenogens of advanced snakes (caenophidians), which is likely the result of the genome-wide homozygosity and the fixation of multiple deleterious alleles and or gene complexes, those that do survive to sexual maturity may have undergone a purging of such deleterious alleles; this alternative outcome may then have a positive effect on an incipient population (see Hedrick, 2007). Evidence supporting this hypothesis is seen in poultry (turkeys and chickens) produced through FP. For over 60 years, FP has been documented in multiple species of galliform birds, but those parthenogen males produced in domesticated chickens and turkeys have shown the greatest levels of viability (Olson and Marsden, 1954; Olson, 1968; Olson et al., 1968; Sarvella, 1973). Successful reproductive performance has been documented in parthenogens of both chickens and turkeys (Cassar et al., 1998). Indeed, while litter sizes of parthenogenetic pitvipers are relatively small, the observation of normal-appearing hemipenes and testes (Reynolds et al., 2012) and apparently viable spermatozoa (W. Booth and G. Schuett, unpubl. data) in natricine snakes suggests promise to a positive outcome in laboratory tests of reproductive competence. Additionally, the capacity for successive parthenogenetic births in caenophidian snakes (Reynolds et al., 2012), and therefore the potential that a viable offspring may be produced by a given female, may offset the initial expenditure; thus, selection for the maintenance of FP as an adaptation in snakes.

Parthenogens produced through FP may also represent valuable tools in the genomic investigations of sex chromosome structure and the identification of sex-determining genes in snakes. In contrast to the basal alethinophidians, caenophidian snakes possess heteromorphic sex chromosomes, with a significant reduction observed in the size of the W chromosome (Matsubara et al., 2006; Vicosa et al., 2013). When investigations are then focused on advanced snakes, we find that the sex chromosomes show varying levels of heteromorphism, with those of pitvipers considered amongst the most highly differentiated (Vicosa et al., 2013). Therefore, they are easily identified using basic karyotyping methods (Zimmerman and Kilpatrick, 1973). Even using such relatively primitive chromosome staining methods, variation in the structure of the W chromosome of pitvipers (Crotalinae) has been revealed (Zimmerman and Kilpatrick, 1973).

While no genome is currently published for a rattlesnake species, draft assemblies of whole genome sequence data are available for the Speckled Rattlesnake (*Crotalus pyrrhus*) (National Center for Biotechnology Information [NCBI], BioProject – PRJNA255393), and the Timber Rattlesnake (*C. horridus*) (NCBI, BioProject – PRJNA181087). Additionally, the genome sequencing of the Prairie Rattlesnake (*C. viridis*) is underway (lead investigator – Todd Castoe, University of Texas, Arlington). Such genomes may, therefore, permit an initial inspection of sex chromosome structure. Given the obvious benefits of reduced genomic heterozygosity, the homozygous individuals produced through FP, therefore, provide a unique opportunity to address sex chromosome structure and evolution. Preliminary identification of Z chromosome structure and the localization of genes associated with sex determination may then be confirmed through later studies in rattlesnake species for which parthenogenetic specimens become available. Given the widespread occurrence of numerous pitviper species in private collections and zoological facilities, their availability is likely to increase as a result of the improved recognition of parthenogenetic births following the description of litter characteristics associated with FP (Booth and Schuett, 2011, 2016; W. Booth et al., unpubl. data).

Conclusions

Rattlesnakes clearly have been and continue to be a major model organism for understanding reproduction of vertebrates in the wild (e.g., Brown, 1991; Duvall et al., 1992; Duvall and Schuett, 1997; Schuett et al., 2002, 2004, 2005, 2011, 2013a, b; Taylor et al., 2004; Cardwell, 2008; Clark et al., 2008, 2010, 2011, 2012, 2014; Prival and Schroff, 2012), with studies on Arizona rattlesnakes constituting a large portion of this research. Their large numbers, ease with which they can be studied, and interesting reproductive strategies have made them ideal study organisms for seasoned researchers and new students alike. However, even with a large number of species in Arizona, coupled with the new technologies that allow researchers to peer more deeply into their lives, much more remains to be discovered.

We suggest that the diversity of rattlesnake species and their habitats in Arizona provide excellent opportunities to learn considerably more about rattlesnake reproduction. Nowhere else do so many different species occur sympatrically and syntopically (see Nowak and Schuett, this volume, Syntopy) and in so many different habitat types (see Species Accounts, volume 1; see Campbell and Lamar, 2004; Ernst and Ernst, 2012). In Arizona, future students of rattlesnake biology have a natural laboratory at their fingertips, where they can study sympatric snakes that exhibit different reproductive strategies, compare reproduction along altitudinal, latitudinal, hydric, and thermal gradients, and apply as-yet undeveloped tools to better understand social behavior and reproductive strategies in these snakes. Several areas naturally emerge as candidates for future studies. Experimental manipulation of hormone concentrations would take our knowledge of reproductive endocrinology to the next level by allowing scientists to understand causal relationships between hormones and reproductive events. Long-term studies of reproduction in individual free-ranging snakes over time and or throughout their range will yield a perspective more valuable than “snap-shot” studies of single populations at one moment in time, potentially offering insight into the influence of climate change on snake reproductive biology (**Box 3**).

Presently, the ease with which large numbers of individuals of a population can be genotyped (either through a classical microsat-

Box 3. Global climate change and its effects on reproduction

Over the past 100 years the earth has seen an unprecedented increase in temperatures with an average of 6°C recorded (López-Alcaide and Macip-Rios, 2011). In the latter half of this time period, from the mid-1970's to the present day, the increase in average temperatures has been the greatest recorded in the last 10,000 years (Jones et al., 2001), with future projections of between 3.7°C and 4.8°C (range 2.5 to 7.8°C) further increase by the end of the 21st century (Intergovernmental Panel on Climate Change IPCC, 2014). Concurrently, we are witnessing the expansion of urban and managed environments at an unprecedented rate in almost all geographic regions (United Nations, 2007). Widespread habitat fragmentation is dissecting populations, reducing or eliminating opportunities for dispersal and gene flow, and drastically altering landscapes. Across these landscapes, populations may experience further perturbations due to over-collection, harvesting, or extirpation (Fitch, 1998; Fitzgerald and Painter, 2000; Gibbons et al., 2000; Martin, 2002; Means, 2009). While affecting both endothermic and ectothermic organisms, the impact on the latter may be considerable (Gibbons et al., 2000; Reading et al., 2010; Sinervo et al., 2010), due in part to the potential for a disruption in ecological processes that influence, for example, species range distributions, dispersal, and abundance (Walther et al., 2002; Parmesan and Yohe, 2003; Araújo and Pearson, 2005; Hickling et al., 2006; Penman et al., 2010) and physiological functions such as locomotion, growth, thermal tolerance, and reproduction (Huey and Stephenson, 1979; Beuchat, 1988; Deutsch et al., 2008; Aubret and Shine, 2010).

With few exceptions, reptiles are considered poor dispersers (Gregory et al., 1987; Luiselli and Capizzi, 1997; Araújo and Pearson, 2005), often revealing natal-site fidelity (Brown and Shine, 2007; Lukoschek and Shine, 2012; Pittman et al., 2014). As such, any impact on species distributions due to or influenced by global change that result in habitat fragmentation or population subdivision may potentially threaten long-term population viability through a reduction in population size, an elevation in inbreeding potential, and potentially a resulting increased susceptibility to disease (Gilpin and Soule, 1986; Frankham et al., 2002; Clark et al., 2011). Despite concern, few studies address these in natural populations (Gardner et al., 2007) and even fewer those of crotaline snakes. Clark et al. (2010, 2011) presented convincing evidence of population decline likely driven by the combined effects of habitat degradation, climate change, and disease in a population of *C. horridus*. The studied population exhibited a significant reduction in allelic diversity, and displayed gross phenotypic indicators of inbreeding (pigmentation abnormalities) and reduced individual health, including skin lesions and potentially fevers. Overall, the population was considered to be undergoing a population extinction vortex and thus in urgent need of genetic rescue. Other studies have documented equally depressing findings across a variety of reptiles, including lizards (Delaney et al., 2010), chelonians (Aponte et al., 2003), and snakes (Rosen and Lowe, 1994; Winne et al., 2007; Reading et al., 2010; Breininger et al., 2012). The studies of Clark et al. (2010, 2011) emphasize the detrimental impact of global change on pitviper populations (see Douglas et al., 2016). As such, we recommend the establishment of long-term population monitoring and the implementation of genetic screening on populations of Arizona rattlesnakes currently at risk and those considered stable, thus providing a baseline for future reference.

Box 3. Continued

Undoubtedly, the impact of climate change on the reproductive physiology of rattlesnakes and other pitvipers warrants urgent and thorough study; but again, field studies are lacking (Douglas et al., 2016). Regardless, concerted effort appears to have been made to investigate the impact of temperature variation on physiological process across a variety of reptiles, thus insight may be gained about potential commonalities across species (Beuchat, 1988; Angilletta et al., 2000; Lourdais et al., 2004; Dubey and Shine, 2011; Michel et al., 2013; Rugiere et al., 2013; Du and Shine, 2015). The results have highlighted clear areas of concern, often linked with a shift in phenology (i.e., timing of life-history events) of the target species. While a species may initially adapt through shifting the breeding season earlier, the temperature range experienced between the onset of the reproductive cycle and parturition may be significant (Figure 2).

While the sex of the offspring of snakes is not governed by the body temperature of the gravid female, and thus the fear of climate-induced extinction driving shifts in sex-ratios expressed in numerous lizard and chelonian populations (Nelson et al., 2004; Wapstra et al., 2009; Telemeco et al., 2013) is removed, thermosensitive processes during multiple phases of embryogenesis that influence survival and phenotype (e.g., body size, scalation, locomotor performance) may be affected by an increase in average temperatures (Beuchat, 1988; Lourdais et al., 2004).

It is clear from the literature that specific areas of pitviper research linked to global/climate-induced change are lacking (see Douglas et al., 2009; Schuett et al., 2013b). These include: a) change in the timing of onset of spermatogenesis and vitellogenesis; b) long-term change in hibernation duration and its consequences on reproductive physiology; c) phenological shifts in reproduction; d) embryonic response during thermosensitive processes; e) habitat fragmentation and its impact on reproduction; and f) long-term variation in predator-prey populations and community dynamics and the impact on reproductive success. Undoubtedly, comparable research on other reptiles has proven fruitful; thus, combined with the ease of maintaining rattlesnakes in captivity, and the diversity of species present within Arizona, these avenues of research will likely prove highly informative in the coming years.

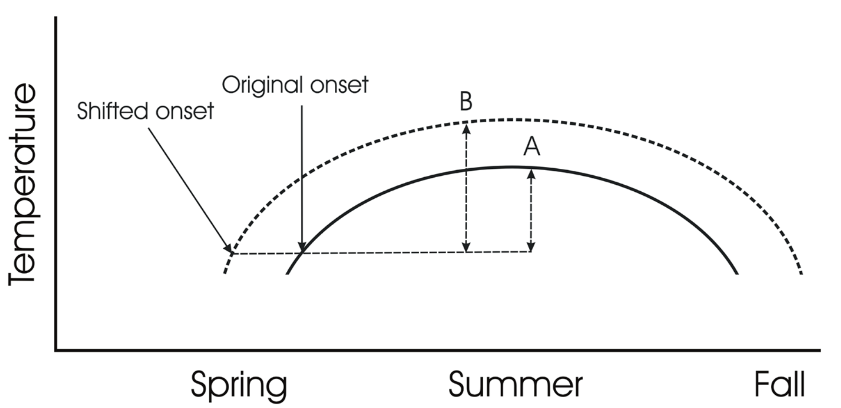

Figure 2. Hypothetical temperature variation experienced across a season (spring – fall) before (solid line) and after (dashed line) a homogeneous temperature increase. Increasing temperatures may shift the onset of seasonal reproduction to the left (i.e., earlier in the season), resulting in a greater variance in temperature range experienced (A = hypothetical temperature range prior; B = post-temperature increase). Modified from Telemeco et al. (2013).

ellite approach or single nucleotide polymorphism detection using next generation methods), often from unorthodox and non-destructively obtained materials (e.g., shed skin, feces, blood), means that reproductive success may be tracked not only across the lives of target individuals, but the lives of their offspring and relatives (Phillips et al., 2008; Tokarska et al., 2009; Weinman et al., 2015). Such information will allow us to understand both within-species lineage-specific variation and among species variation; thus, it will provide valuable insights into the long-term viability of natural populations. Furthermore, upon generating a DNA database for all individuals within target populations, molecular studies in concert with behavioral observations utilizing radio-telemetry will permit a thorough understanding of not only the incidence of multiple paternity in natural populations, but also the prevalence of alternative reproductive strategies such as long-term sperm storage and facultative parthenogenesis. Continued collection that includes both the long-term maintenance in captivity and the preservation of specimens—with tissues and vouchers deposited in natural history museums—is key to the success of future studies (Rocha et al., 2014). In closing, with so many avenues now open to researchers due to the relatively low cost and ease of molecular and behavioral based techniques, research into the reproductive biology of rattlesnakes and other pitvipers is likely to be a proverbial hotbed in the coming years.

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References

Aldridge, R. D. 1992. Oviductal anatomy and seasonal sperm storage in the Southeastern Crowned Snake (*Tantilla coronata*). Copeia 1992: 1,103–1,106.

Almeida-Santos, S. M., I. L. Laporta-Ferreira, M. M. Antoniazzi, and C. Jared. 2004. Sperm storage in males of the snake *Crotalus durissus terrificus* (Crotalinae: Viperidae) in southeastern Brazil. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 139: 169–174.

Almeida-Santos, S. M., and M. G. Salomao. 1997. Long-term sperm storage in the female Neotropical Rattlesnake *Crotalus durissus terrificus* (Viperidae: Crotalinae). Japan. J. Herpetol. 17: 46–52.

Amarello, M. 2012. Social Snakes? Non-Random Association Patterns Detected in a Population of Arizona Black Rattlesnakes (*Crotalus cerberus*). Unpublished thesis, Arizona State University, Tempe, Arizona.

Angilletta Jr, M. J., R. S. Winters, and A. E. Dunham. 2000. Thermal effects on the energetics of lizard embryoes: implications for hatchling phenotypes. Ecology 81: 2,957–2,968.

Aponte, C., G. R. Barreto, and J. Terborgh. 2003. Consequences of habitat fragmentation on age structure and life history in a tortoise population. Biotropica 35: 550–555.

Araújo, M. B., and R. G. Pearson. 2005. Equilibrium of species' distributions with climate. Ecography 28: 693–695.

Arnold, S. J., and D. Duvall. 1994. Animal mating systems: a synthesis based on selection theory. Am. Nat. 143: 317–348.

Ashton, K. G., T. M. Patton, and A. H. Price. 2001. Movement and reproductive biology of female Midget Faded Rattlesnakes, *Crotalus viridis concolor*, in Wyoming. Copeia 2001: 229–234.

Aubret, F., and R. Shine. 2010. Thermal plasticity in young snakes: how will climate change affect the thermoregulatory tactics of ectotherms. J. Exp. Biol. 213: 242–248.

Avise, J. C. 2004. Molecular Markers, Natural History, and Evolution. 2nd edn. Sinauer Associates, Inc., Sunderland, Massachusetts.

Avise, J. C. 2008. Coloniality: The Genetics, Ecology, and Evolution of Sexual Abstinence in Vertebrate Animals. Oxford University Press, Oxford, United Kingdom.

Barros, V. A., L. R. Sueiro, and S. M. Almeida-Santos. 2012. Reproductive biology of the Neotropical Rattlesnake *Crotallus durissus* from northeastern Brazil: a test of phylogenetic conservatism of reproductive patterns. J. Herpetol. 22: 97–104.

Beaupre, S. J. 1995. Comparative ecology of the Mottled Rock Rattlesnake, *Crotalus lepidus*, in Big Bend National Park. Herpetologica 51: 45–56.

Beaupre S. J., and D. Duvall. 1998. Integrative biology of rattlesnakes. BioScience 48: 531–538.

Beaupre, S. J., and H. W. Greene. 2012. Handling hazardous live reptiles. Pp. 130–134 *In* R. W. McDiarmid, M. S. Foster, C. Guyer, J. W. Gibbons, and N. Chernoff (Eds.), Reptile Biodiversity: Standard Methods for Inventory and Monitoring. University of California Press, Los Angeles, California.

Beuchat, C. A. 1988. Temperature effects during gestation in a viviparous lizard. J. Thermal Biol. 13:135–142.

Birkhead, T. 2000. Promiscuity: An Evolutionary History of Sperm Competition. Harvard University Press, Cambridge, Massachusetts.

Birkhead, T., and A. P. Møller. 1993. Sperm Competition and Sexual Selection. Academic Press, London, United Kingdom.

Blackburn, D. G. 1998. Structure, function, and evolution of the oviducts of Squamate reptiles, with special reference to viviparity and placentation. J. Exp. Zool. 282: 560–617.

Blouin-Demers, G., H. L. Gibbs, and P. J. Weatherhead. 2005. Genetic evidence for sexual selection in Black Ratsnakes, *Elaphe obsoleta*. Anim. Behav. 69: 225–234.

Booth, W., and G. W. Schuett. 2011. Molecular genetic evidence for alternative reproductive strategies in North American pitvipers (Serpentes: Viperidae): long-term sperm storage and facultative parthenogenesis. Biol. J. Linn. Soc. 104: 934–942.

Booth, W., and G.W. Schuett. 2016. The emerging phylogenetic patterns of parthenogenesis in snakes. Biol. J. Linn. Soc. 118: 172–186.

Booth, W., D. H. Johnson, S. Moore, C. Schal, and E. L. Vargo. 2011a. Evidence for viable, non-clonal but fatherless boa constrictors. Biol. Lett. 7: 253–256.

Booth, W., L. Million, R. G. Reynolds, G. M. Burghardt, E. L. Vargo, C. Schal, A. C. Tzika, and G. W. Schuett. 2011b. Consecutive virgin births in the New World boid snake, the Colombian Rainbow Boa, *Epicrates maurus*. J. Hered. 102: 759–763.

Booth, W., G. W. Schuett, A. Ridgway, D. W. Buxton, T. A. Castoe, G. Bastone, C. Bennett, and W. McMahan. 2014. New insights on facultative parthenogenesis in pythons. Biol. J. Linn. Soc. 112: 461–468.

Booth, W., C. F. Smith, P. H. Eskridge, S. K. Hoss, J. R. Mendelson III, and G. W. Schuett. 2012. Facultative parthenogenesis discovered in wild vertebrates. Biol. Lett. 8: 983–985.

Breininger, D. R., M. J. Mazerolle, M. R. Bolt, M. L. Legare, J. H. Drese, and J. E. Hines. 2012. Habitat fragmentation effects on annual survival of the federally protected Eastern Indigo Snake. Anim. Conserv. 15: 361–368.

Bronikowski, A. M., and S. J. Arnold. 1999. The evolutionary ecology of life history variation in the Garter Snake *Thamnophis elegans*. Ecology 80: 2,314–2,325.

Brown, G. P., and R. Shine. 2007. Like mother, like daughter: inheritance of nest-site location in snakes. Biol Lett 3: 131–133.

Brown, W. S. 1991. Female reproductive ecology in a northern population of the Timber Rattlesnake, *Crotalus horridus*. Herpetologica 47: 101–115.

Bryson, R. W., C. W. Linkem, M. E. Dorcas, A. Lathrop, J. M. Jones, J. Alvarado-Diaz, C. I. Grunwald, and R. W. Murphy. 2014. Multilocus species delimination in the *Crotalus triseriatus* species group (Serpentes: Viperidae: Crotalinae), with the description of two new species. Zootaxa 3826: 475–496.

Camazine, B., W. Garstka, R. Tokarz, and D. Crews. 1980. Effects of castration and androgen replacement on male courtship behavior in the Red-sided Garter Snake (*Thamnophis sirtalis parietalis*). Horm. Behav. 14: 358–372.

Campbell, J. A., and W. W. Lamar. 2004. The Venomous Reptiles of the Western Hemisphere, 2 vols. Cornell University Press, Ithaca, New York

Cardwell, M. D. 2008. The reproductive ecology of Mohave Rattlesnakes. J. Zool. 274: 65–76.

Carpenter, C. C. 1952. Comparative ecology of the Common Garter Snake (*Thamnophis s. sirtalis*), the Ribbon Snake (*Thamnophis s. sauritus*), and Butler's Garter Snake (*Thamnophis butleri*) in mixed populations. Ecol. Monogr. 22: 235–258.

Cassar, G., T. M. John, and R. J. Etches. 1998. Observations on ploidy of cells on reproductive performance in parthenogenetic turkeys. Poult. Sci. 77: 1,457–1,462.

Castoe, T. A., A. M. Bronikowski, E. D. Brodie III, S. V. Edwards, M. E. Pfrender, M. D. Shapiro, D. D. Pollock, and W. C. Warren. 2011. A proposal to sequence the genome of a Garter Snake (*Thamnophis sirtalis*). Standards in Genomic Science 4: 257–270.

Castoe, T. A., A. W. Poole, A. P. J. de Koning, K. L. Jones, D. F. Tomback, S. J. Oyler-McCance, J. A. Fike, S. L. Lance, J. W. Streicher, E. N. Smith, and D. D. Pollock. 2012. Rapid microsatellite identification from illumina paired-end genomic sequencing in two birds and a snake. PLoS One 7: e30953.

Castoe, T. A., A. W. Poole, W. Gu, A. P. J. de Koning, J. M. Daza, E. N. Smith, and D. D. Pollock. 2010. Rapid identification of thousands of Copperhead snake (*Agkistrodon contortrix*) microsatellite loci from modest amounts of 454 shotgun genome sequence. Mol. Ecol. Resour. 10: 341–347.

Clark, R. W., W. S. Brown, R. Stechert, and K. R. Zamundio. 2010. Roads, interrupted dispersal, and genetic diversity in Timber Rattlesnakes. Conserv. Biol. 24: 1,059–1,069.

Clark, R. W., W. S. Brown, R. Stechert, and H. W. Greene. 2012. Cryptic sociality in rattlesnakes (*Crotalus horridus*) detected by kinship analysis. Biol. Lett. 8: 523–525.

Clark, R. W., W. S. Brown, R. Stechert, and K. R. Zamudio. 2008. Integrating individual behaviour and landscape genetics: the population structure of Timber Rattlesnake hibernacula. Mol. Ecol. 17: 719–730.

Clark, R. W., M. N. Marchand, B. J. Clifford, R. Stechert, and S. Stephens. 2011. Decline of an isolated Timber Rattlesnake (*Crotalus horridus*) population: interactions between climate change, disease, and loss of genetic diversity. Biol. Conserv. 144: 886–891.

Clark, R. W., G. W. Schuett, R. A. Repp, M. Amarello, C. F. Smith, and H.-W. Herrmann. 2014. Mating systems, reproductive success, and sexual selection in secretive species: a case study of the Western Diamond-backed Rattlesnake, *Crotalus atrox*. PLoS One 9: e90616.

Crews, D. 1984. Gamete production, sex hormone secretion, and mating behavior uncoupled. Horm. Behav. 18: 22–28.

Crews, D., B. Camazine, M. Diamond, R. Mason, R. R. Tokarz, and W. R. Garstka. 1984. Hormonal independence of courtship behavior in the male Garter Snake. Horm. Behav. 18: 29–41.

Crews, D., M. Grassman, and J. Lindzey. 1986. Behavioral facilitation of reproduction in sexual and unisexual whiptail lizards. Proc. Natl. Acad. Sci. USA 83: 9,547–9,550.

Custodia-Lora, N., and I. P. Callard. 2002. Progesterone and progesterone receptors in reptiles. Gen. Comp. Endocrinol. 127: 1–7.

Davenport, M. 1995. Evidence of possible sperm storage in the Caiman, *Paleosuchus palpebrosus*. Herpetol. Rev. 26: 14–15.

Dawley, R. M., and J. P. Bogart. 1989. An introduction to unisexual vertebrates. Pp. 1–18 *In* R. M. Dawley and J. P. Bogart (Eds.), Evolution and Ecology of Unisexual Vertebrates. Bulletin of the New York State Museum, Albany, New York.

Delaney, K. S., S. P. D. Riley, and R. N. Fisher. 2010. A rapid, strong, and convergent genetic response to urban habitat fragmentation in four divergent and widespread vertebrates. PLoS One 5: e12767.

DeNardo, D. F., and E. N. Taylor. 2011. Hormones and reproduction in free-ranging snakes. Pp. 265–287 *In* D. M. Sever and R. D. Aldridge (Eds.), Reproductive Biology and Phylogeny of Snakes. CRC Press (Taylor & Francis Group), Boca Raton, Florida.

Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Nat. Acad. Sci. USA 105: 6,668–6,672.

Devine, M. C. 1977. Copulatory plugs, restricted mating opportunities and reproductive competition among male Garter Snakes. Nature 267: 345–346.

Devine, M. C. 1984. Potential for sperm competition in reptiles: behavioral and physiological consequences. Pp. 509–521 *In* R. L. Smith (Ed.). Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, New York, New York.

Diller, L. V., and R. L. Wallace. 1984. Reproductive biology of the Northern Pacific Rattlesnake (*Crotalus viridis oreganus*) in northern Idaho. Herpetologica 40: 182–193.

Douglas, M. E., M. R. Douglas, G. W. Schuett, L. W. Porras, and A. T. Holycross. 2002. Phylogeography of the western rattlesnake (*Crotalus viridis*) complex, with emphasis on the Colorado Plateau. Pp. 11–50 *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.), Biology of the Vipers. Eagle Mountain Publishing, LC, Eagle Mountain, Utah.

Douglas, M. E., M. R. Douglas, G. W. Schuett, and L. W. Porras. 2009. Climate change and evolution of the New World pitviper genus *Agkistrodon* (Viperidae). J. Biogeogr. 36: 1,164–1,180.

Douglas, M. R., M. A. Davis, M. Amarello, J. J. Smith, G. W. Schuett, H.-W. Herrmann, and M. E. Douglas. 2016. Anthropogenic impacts drive conservation and ecosystem metrics of a niche conserved rattlesnake on the Colorado Plateau of Western North America. Royal Soc. Open Sci. 3: 160047.

Du, W., and R. Shine. 2015. The behavioural and physiological strategies of bird and reptile embryos in response to unpredictable variation in nest temperature. Biol. Rev. 90: 19–30.

Dubach, J., A. Sajewicz, and R. Pawley. 1997. Parthenogenesis in the Arafuran Filesnake (*Acrochordus arafurae*). Herpetol. Nat. Hist. 5: 11–18.

Dubey, S., G. P. Brown, T. Madsen, and R. Shine. 2009. Sexual selection favours large body size in males of a tropical snake (*Stegonotus cucullatus*, Colubridae). Anim. Behav. 77: 177–182.

Dubey, S., and R. Shine. 2011. Predicting the effects of climate change on reproductive fitness of an endangered montane lizard, *Eulamprus leuraensis* (Scincidae). Climate Change 10: 531–547.

Duvall, D., and G. W. Schuett. 1997. Straight-line movement and competitive mate searching in Prairie Rattlesnakes, *Crotalus viridis viridis*. Anim. Behav. 54: 329–334.

Duvall, D., S. J. Arnold, and G. W. Schuett. 1992. Pitviper mating systems: ecological potential, sexual selection, and microevolution. Pp. 321-336 *In* J. A. Campbell and E. D. Brodie Jr. (Eds.), Biology of the Pitvipers. Selva, Tyler, Texas.

Duvall, D., G. W. Schuett, and S. J. Arnold. 1993. Ecology and evolution of snake mating systems. Pp. 165–200 *In* R. A. Seigel and J. T. Collins (Eds.). Snakes. Ecology and Behavior. McGraw Hill, New York, New York.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197: 215–223.

Ernst, C. H., and E. M. Ernst. 2012. Venomous Reptiles of the United States, Canada, and Northern Mexico, 2 vols. Johns Hopkins University Press, Baltimore, Maryland.

Fitch, H. S. 1998. The Sharon Springs roundup and Prairie Rattlesnake demography. Trans. Kans. Acad. Sci. 101: 101113.

Fitzgerald, K. T., B. K. Shipley, K. L. Newquist, R. Vera, and A. A. Flood. 2013. Additional observations and notes on the natural history of the Prairie Rattlesnake (*Crotalus viridis*) in Colorado. Topics in Companion Animal Medicine 28: 167–176.

Fitzgerald, L. A., and C. W. Painter. 2000. Rattlesnake commercialization: long-term trends, issues, and implications for conservation. Wildlife Soc. Bull. 28: 235–253.

Ford, N. B., and J. P. Karges. 1987. Reproduction in the Checkered Garter Snake, *Thamnophis marcianus* in New Mexico. Southwest. Nat. 32: 93–101.

Fox, W. 1963. Special tubules for sperm storage in female lizards. Nature 198: 500–501.

Fox, W., and H. C. Dessauer. 1962. The single right oviduct and other urogenital structures of female *Typhlops* and *Leptotyphlops*. Copeia 1962: 590–597.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge, United Kingdom.

Friesen, C. R., R. Shine, R. W. Krohmer, and R. T. Mason. 2013. Not just a chastity belt: the functional significance of mating plugs in Garter Snakes, revisited. Biol. J. Linn. Soc. 109: 893–907.

Friesen, C., A. Kerns, and R. Mason. 2014a. Factors influencing paternity in multiply mated female Red-sided Garter Snakes and the persistent use of sperm stored over winter. Behav. Ecol. Sociobiol. 68: 1,419–1,430.

Friesen, C. R., M. K. Squire, and R. T. Mason. 2014b. Intrapopulational variation of ejaculate traits and sperm depletion in Red-sided Garter Snakes. J. Zool. 292: 192–201.

Gardner, T. A., J. Barlow, and C. A. Peres. 2007. Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. Biol. Conserv. 138: 166–179.

Garner, T. W. J., P. T. Gregory, G. F. McCracken, G. M. Burghardt, B. F. Koop, S. E. McLain, R. J. Nelson, and J. D. McEachran. 2002. Geographic variation of multiple paternity in the Common Garter Snake (*Thamnophis sirtalis*). Copeia 2002: 15–23.

Garner, T. W. J., and K. W. Larsen. 2005. Multiple paternity in the Western Terrestrial Garter Snake, *Thamnophis elegans*. Can. J. Zool. 83: 656–663.

Garstka, W. R., B. Camazine, and D. Crews. 1982. Interactions of behavior and physiology during the annual reproductive cycle of the Red-sided Garter Snake (*Thamnophis sirtalis parietalis*). Herpetologica 38: 104–123.

Germano, D. J., and P. T. Smith. 2010. Molecular evidence for parthenogenesis in the Sierra Garter Snake, *Thamnophis couchii* (Colubridae). Southwest. Nat. 55: 280–282.

Gibbons, W. J. 1972. Reproduction, growth, and sexual dimorphism in the Canebrake Rattlesnake (*Crotalus horridus atricaudatus*). Copeia 1972: 222–226.

Gibbons, W. J., D. E. Scott, T. J. Ryan, K. A. Buhlmann, T. D. Tuberville, B. S. Metts, J. L. Greene, T. Mills, Y. Leiden, S. Poppy, and C. Winne. 2000. The global decline of reptiles, deja vu amphibians. BioScience 50: 653–666.

Gibbs, H. L., and P. J. Weatherhead. 2001. Insights into population ecology and sexual selection in snakes through the application of DNA-based genetic markers. J. Hered. 92: 173–179.

Gienger, C. M., and D. D. Beck. 2011. Northern Pacific Rattlesnakes (*Crotalus oreganus*) use thermal and structural cues to choose overwintering hibernacula. Can. J. Zool. 89: 1,084–1,090.

Gilpin, M., and M. Soule. 1986. Minimum viable populations: the process of species extinction. Pp. 19-34 *In* M. E. Soule and G. H. Orians (Eds.), Conservation Biology: The Science of Scarcity and Diversity. Sinauer Associates, Sunderland, Massachusetts.

Gist, D. H., and J. M. Jones. 1989. Sperm storage within the oviduct of turtles. J. Morphol. 199: 379–384.

Glaudas, X., S. R. Goldberg, and B. T. Hamilton. 2009. Timing of reproduction of a cold desert viperid snake from North America, the Great Basin Rattlesnake (*Crotalus lutosus*). J. Arid. Environ. 73: 719–725.

Goldberg, S. R. 1999a. Reproduction in the Blacktail Rattlesnake, *Crotalus molossus* (Serpentes: Viperidae). Tex. J. Sci. 51: 323–328.

Goldberg, S. R. 1999b. Reproduction in the Tiger Rattlesnake, *Crotalus tigris* (Serpentes: Viperidae). The Tex. J. Sci. 51: 31–36.

Goldberg, S. R. 2000. Reproduction in the Twin-spotted Rattlesnake, *Crotalus pricei* (Serpentes: Viperidae). Western North Amer. Nat. 60: 98–100.

Goldberg, S. R. 2002. Reproduction in the Arizona Black Rattlesnake, *Crotalus viridis cerberus* (Viperidae). Herpetol. Nat. Hist. 9: 75–78.

Goldberg, S. R. 2007. Testicular cycle of the Western Diamondback Rattlesnake, *Crotalus atrox* (Serpentes: Viperidae), from Arizona. Bull. Maryland Herpetol. Soc. 43: 103–107.

Goldberg, S. R., and A. T. Holycross. 1999. Reproduction in the Desert Massasauga, *Sistrurus catenatus edwardsii*, in Arizona and Colorado. Southwest. Nat. 44: 531–535.

Goldberg, S. R., and P. C. Rosen. 2000. Reproduction in the Mojave Rattlesnake, *Crotalus scutulatus* (Serpentes: Viperidae). Tex. J. Sci. 52: 101–109.

Graham, S.P., R. L. Earley, S. K. Hoss, G. W. Schuett, and M. S. Grober. 2008. The reproductive biology of male Cottonmouths (*Agkistrodon piscivorus*): do plasma steroid hormones predict the mating season? Gen. Comp. Endocrinol. 159: 226–235.

Graves, B. M., and D. Duvall. 1993. Reproduction, rookery use, and thermoregulation in free-ranging, pregnant *Crotalus v. viridis*. J. Herpetol. 27: 33–41.

Greene, H. W., P. G. May, D. L. Hardy Sr, J. M. Sciturro, and T. M. Farrell. 2002. Parental behavior in vipers. Pp. 178–206 *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.), Biology of the Vipers. Eagle Mountain Publishing, LC, Eagle Mountain, Utah.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Anim. Behav. 28: 1,140–1,162.

Gregory, P. T. 1977. Life history observations of three species of snakes in Manitoba. Can. Field. Nat. 91: 19–27.

Gregory, P. T., and K. W. Larsen. 1993. Geographic variation in reproductive characteristics among Canadian populations of the Common Garter Snake (*Thamnophis sirtalis*). Copeia 1993: 946–958.

Gregory, P. T., J. M. Macartney, and K. W. Larsen. 1987. Spatial patterns and movements. Pp. 366–395 *In* R. A. Seigel, J. T. Collins, and S. S. Novak (Eds.), Snakes. Ecology and Evolutionary Biology. McGraw Hill, New York, New York.

Griffith, S. C., I. P. F. Owens, and K. A. Thuman. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol. Ecol. 11: 2,195-2,212.

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

Haines, T. P. 1940. Delayed fertilzation in *Leptodeira annulata polysticta*. Copeia 1940: 116–118.

Halpert, A. P., W. R. Garstka, and D. Crews. 1982. Sperm transport and storage and its relation to the annual sexual cycle of the female Red-sided Garter Snake, *Thamnophis sirtalis parietalis*. J. Morphol. 174: 149–159.

Hedrick, P. W. 2007. Virgin birth, genetic variation and inbreeding. Biol. Lett. 3: 715–716.

Hickling, R., D. B. Roy, J. K. Hill, R. Fox, and C. D. Thomas. 2006. The distributions of a wide range of taxonomic groups are expanding polewards. Global Change Biology 12: 450–455.

Ho, S. M., S. Kleis, R. McPherson, G. J. Heisermann, and I. P. Callard. 1982. Regulation of vitellogenesis in reptiles. Herpetologica 38: 40–50.

Höggren, M., and H. Tegelström. 1995. DNA fingerprinting shows within-season multiple paternity in the Adder (*Vipera berus*). Copeia 1995: 271–277.

Holding, M. L., J. A. Frazier, S. W. Dorr, S. N. Henningsen, I. T. Moore, and E. N. Taylor. 2014a. Physiological and behavioral effects of repeated handling and short-distance translocations on free-ranging Northern Pacific rattlesnakes (*Crotalus oreganus oreganus*). J. Herpetol. 48: 233–239.

Holding, M. L., J. A. Frazier, S. W. Dorr, N. B. Pollock, P. J. Muellenman, A. Branske, S. N. Henningsen, C. Eikenaar, C. Escallón, C. E. Montgomery, I. T. Moore, and E. N. Taylor. 2014b. Wet- and dry-season steroid hormone profiles and stress reactivity of an insular dwarf snake, the Hog Island Boa (*Boa constrictor imperator*). Physiol. Biochem. Zool. 87: 363–373.

Holt, W. V., and R. E. Lloyd. 2010. Sperm storage in the vertebrate female reproductive tract: how does it work so well? Theriogenology 73: 713–722.

Holycross, A. T., and S. R. Goldberg. 2001. Reproduction in northern populations of the Ridgenose Rattlesnake, *Crotalus willardi* (Serpentes: Viperidae). Copeia 2001: 473–481.

Hoss, S. K., D. Deutschman, W. Booth, and R. W. Clark. 2015. Post-birth separation affects the affiliative behavior of kin in a pitviper with maternal attendance. Biol. J. Linn. Soc. 116: 637–648.

Hoss, S. K., M. J. Garcia, R. L. Earley, and R. W. Clark. 2014. Fine-scale hormonal patterns associated with birth and maternal care in the Cottonmouth (*Agkistrodon piscivorus*), a North American pitviper snake. Gen. Comp. Endocrinol. 208: 85–93.

Huey, R. B., and R. D. Stevenson. 1979. Integrating thermal physiology and ecology of ectotherms: discussion of approaches. Amer. Zool. 19: 357–366.

Johnston, E. E., M. S. Rand, and S. G. Zweifel. 2006. Detection of multiple paternity and sperm storage in a captive colony of the central Asian Tortoise, *Testudo horsfieldii*. Can. J. Zool. 84: 520–526.

Jones, P. D., T. J. Osborn, and K. R. Briffa. 2001. The evolution of climate over the last millennium. Science 292: 662–667.

Jordan, M. A., N. Perrine-Ripplinger, and E. T. Carter. 2015. An independent observation of facultative parthenogenesis in the Copperhead (*Agkistrodon contortrix*). J. Herpetol. 49: 118–121.

Kinney, M. E., R. F. Wack, R. A. Grahn, and L. Lyons. 2013. Parthenogenesis in a Brazilian Rainbow Boa (*Epicrates cenchria cendhria*). Zoo Biol. 32: 172–176.

Kissner, K. J., P. J. Weatherhead, and H. L. Gibbs. 2005. Experimental assessment of ecological and phenotypic factors affecting male mating success and polyandry in Northern Watersnakes, *Nerodia sipedon*. Behav. Ecol. Sociobiol. 59: 207–214.

Klauber, L. M. 1972. Rattlesnakes. Their Habits, Life Histories, and Influence on Mankind, 2 vols., 2nd edn. University of California Press, Berkeley and Los Angeles, California.

Krohmer, R. W. 2004. The male Red-sided Garter Snake (*Thamnophis sirtalis parietalis*): reproductive pattern and behavior. ILAR Journal 45: 65–74.

Krohmer, R. W., M. Grassman, and D. Crews. 1987. Annual reproductive cycle in the male Red-sided Garter Snake, *Thamnophis sirtalis parietalis*: field and laboratory studies. Gen. Comp. Endocrinol. 68: 64–75.

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

Levine, B. A. 2013. Genetic Structure of the Copperhead (Viperidae: *Agkistrodon contortrix mokasen*) at its Most Northern Distribution. Unpublished thesis, University of Arkansas, Fayetteville, Arkansas.

Levine, B A., C. F. Smith, G. W. Schuett, M. R. Douglas, M. A. Davis. and M. E. Douglas. 2015. Bateman-Trivers in the 21st century: sexual selection in a North American pitviper. Biol. J. Linn. Soc. 114: 436–445.

Levine, B. A., C. F. Smith, M. R. Douglas, M. A. Davis, G. W. Schuett, S. J. Beaupre, and M. E. Douglas. 2016. Population genetics of the Copperhead at its most northeastern distribution. Copeia 2016: 448–457.

Lind, C. M., and S. J. Beaupre. 2014. Natural variation in steroid hormone profiles of male Timber Rattlesnakes, *Crotalus horridus*, in northwest Arkansas. Gen. Comp. Endocrinol. 206: 72–79.

Lind, C. M., B. Flack, D. D. Rhoads, and S. J. Beaupre. 2016. The mating system and reproductive life history of female Timber Rattlesnakes in northwestern Arkansas. Copeia 2016: 518–528.

Lourdais, O., R. Shine, X. Bonnet, M. Guillon, and G. Naulleau. 2004. Climate affects embryonic development in a viviparous snake, *Vipera aspis*. Oikos 104: 551–560.

Ludwig, M., and H. Rahn. 1943. Sperm storage and copulatory adjustment in the Prairie Rattlesnake. Copeia 1943: 15–18.

Luiselli, L., and D. Capizzi. 1997. Influences of area, isolation and habitat features on distribution of snakes in Mediterranean fragmented woodlands. Biodivers. Conserv. 6: 1,339–1,351.

Lukoschek, V., and R. Shine. 2012. Sea snakes rarely venture far from home. Ecol. Evol. 2: 1,113–1,121.

Lutterschmidt, D. I., M. P. LeMaster, and R. T. Mason. 2006. Minimal overwintering temperatures of Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*): a possible cue for emergence? Can. J. Zool. 84: 771–777.

Lutterschmidt, D. I., and R. T. Mason. 2009. Endocrine mechanisms mediating temperature-induced reproductive behavior in Red-sided Garter Snakes (*Thamnophis sirtalis parietalis*). J. Exp. Biol. 212: 3,108-3,118.

López-Alcaide, S., and R. Macip-Rios. 2011. Effects of climate change in amphibians and reptiles. Pp. 163–184 *In* O. Grillo and G. Venora (Eds.), Biodiversity Loss in a Changing World. InTech, Hampshire, England.

Macartney, J. M., and P. T. Gregory. 1988. Reproductive biology of female rattlesnakes (*Crotalus viridis*) in British Columbia. Copeia 1988: 47–57.

Macartney, J. M., P. T. Gregory, and K. W. Larsen. 1988. A tabular survey of data on movements and home ranges of snakes. J. Herpetol. 22:61-73.

Madsen, T., B. Ujvari, M. Olsson, and R. Shine. 2005. Paternal alleles enhance female reproductive success in tropical pythons. Mol. Ecol. 14: 1,783–1,787.

Marinho, C. E., S. M. Almeida-Santos, S. C. Yamasaki, and P. F. Silveira. 2009. Peptidase activities in the semen from the ductus deferens and uterus of the Neotropical Rattlesnake *Crotalus durissus terrificus*. J. Comp. Physiol. 179: 635–664.

Martin, W. H. 1993. Reproduction of the Timber Rattlesnake (*Crotalus horridus*) in the Appalachian Mountains. J. Herpetol. 27: 133–143.

Martin, W. H. 2002. Life history constraints on the Timber Rattlesnake (*Crotalus horridus*) at its climatic limits. Pp. 285–306 *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.). Biology of the Vipers, Eagle Mountain Publishing, LC, Eagle Mountain, Utah.

Matsubara, K., H. Tanui, M. Toriba, K. Yamada, C. Nishida-Umehara, K. Agata, and Y. Matsuba. 2006. Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake chromosomes. Proc. Natl. Acad. Sci. USA. 103: 18,190–18,195.

McCracken, G. F., G. M. Burghardt, and S. E. Houts. 1999. Microsatellite markers and multiple paternity in the Garter Snake *Thamnophis sirtalis*. Mol. Ecol. 8: 1,475–1,479.

Mead, R. A., V. P. Eroschenko, and D. R. Highfill. 1981. Effects of progesterone and estrogen on the histology of the oviduct of the Garter Snake, *Thamnophis elegans*. Gen. Comp. Endocrinol. 45: 345–354.

Means, D. B. 2009. Effects of rattlesnake roundups on the Eastern Diamondback Rattlesnake (*Crotalus adamanteus*). Herpetol. Conserv. Biol. 4: 132–141.

Michel, C. L., J-H. Pastore, and X. Bonnet. 2013. Impact of cool versus warm temperatures on gestation in the Aspic Viper (*Vipera aspis*). Comp. Biochem. Physiol. A. 165: 338–342.

Møller, A. P., and J. J. Cuervo. 2000. The evolution of paternity and paternal care in birds. Behav. Ecol. 11: 472–485.

Moore, I. T., and T. S. Jessop. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Horm. Behav. 43: 39–47.

Moore, I. T., J. P. Lerner, D. T. Lerner, and R. T. Mason. 2000. Relationships between annual cycles of testosterone, corticosterone, and body condition in male Red-spotted Garter Snakes, *Thamnophis sirtalis concinnus*. Physiol. Biochem. Zool. 73: 307–312.

Murphy, J. B., and B. L. Armstrong. 1978. Maintenance of Rattlesnakes in Captivity. Univ. Kansas Publ. Mus. Nat Hist. Special Publ. No. 3.

Murphy, R. W., J. Fu, R. D. Macculloch, I. S. Darevsky, and L. A. Kupriyanova. 2000. A fine line between sex and unisexuality: the phylogenetic constraints on parthenogenesis in lacertid lizards. Zool. J. Linn. Soc. 130: 527–549.

Olsen, M. W. 1975. Avian parthenogenesis. USDA ARS-NE: 1–85.

Nelson, N. J., M. B. Thompson, S. Pledger, S. N. Keall, and C. H. Daugherty. 2004. Do TSD sex ratios, and nest characteristics influence the vulnerability of Tuatara global warming? International Congress Series 1275: 250–257.

Nowak, E. M., T. C. Theimer, and G. W. Schuett. 2008. Functional and numerical responses of predators: where do vipers fit in the traditional paradigms? Biol. Rev. 83: 601–620.

Nunney, L. 1993. The influence of mating system and overlapping generations on effective population size. Evolution 47: 1,329–1,341.

Nussbaum, R. A. 1980. The Brahminy Blind Snake (*Ramphlotyphlops braminus*) in the Seychelles archeplago: distribution, variation, and further evidence for parthenogenesis. Herpetologica 36: 215–221.

Olsen, M. W. 1968. Changes in level of parthenogenetic development in turkey eggs during two testing seasons. Poultry Science 47: 2,015-2,016.

Olsen, M. W., and S. J. Marsden. 1954. Natural parthenogenesis in turkey eggs. Science 120: 545–546.

Olsen, M. W., S. O. Wilson, and H. L. Marks. 1968. Genetic control in parthenogenesis in chickens. J. Hered. 59: 41–42.

Olsson, M., and T. Madsen. 1998. Sexual selection and sperm competition in reptiles. Pp. 503–564 *In* T. Birkhead and A. P. Møller (Eds.), Sperm Competition and Sexual Selection. Academic Press, San Diego, California.

O'Shea, M., S. Slater, R. S. Scott, S. Smith, K. McDonald, B. Lawrence. and M. Kubiak. 2016. Herpetoculture Notes. *Eunectes murinus* (Green Anaconda). Reproduction/facultative parthenogenesis. Herpetol. Rev. 47: 73.

Ota, H., T. Hikida, M. Matsui, A. Mori, and A. H. Wynn. 1991. Morphological variation, karyotype and reproduction of the parthenogenetic Blind Snake, *Ramphotyphlops braminus*, from the insular region of East Asia and Saipan. Amphibia-Reptilia 12: 181–193.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421: 37–42.

Pearse, D. E., and J. C. Avise. 2001. Turtle mating systems: behavior, sperm storage, and genetic paternity. J. Hered. 92: 206–211.

Pearse, D. E., F. J. Janzen, and J. C. Avise. 2001. Genetic markers substantiate long-term sperm storage and utilization of sperm by female Painted Turtles. Heredity 86: 378–384.

Penman, T. D., D. A. Pike, J. K. Webb, and R. Shine. 2010. Predicting the impact of climate change on Australia's most endangered snake, *Hoplocephalus bungaroides*. Diversity and Distributons 16: 109–118.

Perkins, M. J., and B. D. Palmer. 1996. Histology and functional morphology of the oviduct of an oviparous snake, *Diadophis punctatus*. J. Morphol. 227:67–79.

Petrie, M., and B. Kempenaers. 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends Ecol. Evol. 13: 52–58.

Phillips, C., M. Fondevila, M. García-Magariños, A. Rodriguez, A. Salas, Á. Carracedo, and M. V. Lareu. 2008. Resolving relationship tests that show ambiguous STR results using autosomal SNPs as supplementary markers. Forensic Science International: Genetics 2: 198–204.

Pittman, S. E., K. M. Hart, M. S. Cherkiss, R. W. Snow, I. Fujisaki, B. J. Smith, F. J. Mazzotti, and M. E. Dorcas. 2014. Homing of invasive Burmese Pythons in South Florida: evidence for map and compass senses in snakes. Biol. Lett. 10: 20140040.

Portnoy, D. S., C. M. Hollenbeck, J. S. Johnston, H. M. Casman, and J. R. Gold. 2014. Parthenogenesis in a Whitetip Reef Shark *Triaenodon obesus* involves a reduction in ploidy. J. Fish Biol. 85: 502–508.

Pozarowski, K., J. Ivy, and H.-W. Herrmann. 2013. Using rattlesnake microsatellites to determine paternity in captive Bushmasters (*Lachesis muta*). Zoo Biol. 32: 454–456.

Prival, D. B., and M. J. Schroff. 2012. A 13-year study of a northern population of Twin-spotted Rattlesnakes (*Crotalus pricei*): growth, reproduction, survival, and conservation. Herpetol. Monogr. 26: 1–18.

Prival, D. B., M. J. Goode, E. S. Don, C. R. Schwalbe, and M. J. Schroff. 2002. Natural history of a northern population of Twin-spotted Rattlesnakes, *Crotalus pricei*. J. Herpetol. 36: 598–607.

Prosser, M. R., P. J. Weatherhead, H. L. Gibbs, and G. P. Brown. 2002. Genetic analysis of the mating system and opportunity for sexual selection in Northern Water Snakes (*Nerodia sipedon*). Behav. Ecol. 13: 800–807.

Pyron, R. A., F. T. Burbrink, and J. J. Weins. 2013. A phylogeny and revised classification of Squamata, including 4,161 species of lizards and snakes. BMC Evol. Biol. 13: 93.

Rafajlović, M., A. Eriksson, A. Rimark, S. Hintz-Saltin, G. Charrier, M. Panova, C. André, K. Johannesson, and B. Mehlig. 2013. The effect of multiple paternity on genetic diversity of small populations during and after colonisation. PLoS One 8: e75587.

Reading, C. J., L. M. Luiselli, G. C. Akani, X. Bonnet, G. Amori, J. M. Ballouard, E. Filippi, G. Naulleau, D. Pearson, and L. Rugiero. 2010. Are snake populations in widespread decline. Biol. Lett. 6: 777–780.

Reynolds, R. G., W. Booth, G. W. Schuett, B. M. Fitzpatrick, and G. M. Burghardt. 2012. Successive virgin births of viable male progeny in the Checkered Gartersnake, *Thamnophis marcianus*. Biol. J. Linn. Soc. 107: 566–572.

Rocha, L. A., A. Aleixo, G. Allen, F. Almeda, C. C. Baldwin, M. V. L. Barclay, J. M. Bates, A. M. Bauer, F. Benzoni, C. M. Berns, M. L. Berumen, D. C. Blackburn, S. Blum, F. Bolaños, R. C. K. Bowie, R. Britz, R. M. Brown, C. D. Cadena, K. Carpenter, L. M. Ceríaco, P. Chakrabarty, G. Chaves, J. H. Choat, K. D. Clements, B. B. Collette, A. Collins, J. Coyne, J. Cracraft, T. Daniel, M. R. de Carvalho, K. de Queiroz, F. Di Dario, R. Drewes, J. P. Dumbacher, A. Engilis, M. V. Erdmann, W. Eschmeyer, C. R. Feldman, B. L. Fisher, J. Fjeldså, P. W. Fritsch, J. Fuchs, A. Getahun, A. Gill, M. Gomon, T. Gosliner, G. R. Graves, C. E. Griswold, R. Guralnick, K. Hartel, K. M. Helgen, H. Ho, D. T. Iskandar, T. Iwamoto, Z. Jaafar, H. F. James, D. Johnson, D. Kavanaugh, N. Knowlton, E. Lacey, H. K. Larson, P. Last, J. M. Leis, H. Lessios, J. Liebherr, M. Lowman, D. L. Mahler, V. Mamonekene, K. Matsuura, G. C. Mayer, H. Mays, J. McCosker, R. W. McDiarmid, J. McGuire, M. J. Miller, R. Mooi, R. D. Mooi, C. Moritz, P. Myers, M. W. Nachman, R. A. Nussbaum, D. Ó. Foighil, L. R. Parenti, J. F. Parham, E. Paul, G. Paulay, J. Pérez-Emán, A. Pérez-Matus, S. Poe, J. Pogonoski, D. L. Rabosky, J. E. Randall, J. D. Reimer, D. R. Robertson, M-O. Rödel, M. T. Rodrigues, P. Roopnarine, L. Rüber, M. J. Ryan, F. Sheldon, G. Shinohara, A. Short, W. B. Simison, W. F. Smith-Vaniz, V. G. Springer, M. Stiassny, J. G. Tello, C. W. Thompson, T. Trnski, P. Tucker, T. Valqui, M. Vecchione, E. Verheyen, P. C. Wainwright, T. A. Wheeler, W. T. White, K. Will, J. T. Williams, G. Williams, E. O. Wilson, K. Winker, R. Winterbottom, and C. C. Witt. 2014. Specimen collection: an essential tool. Science 344: 814–815.

Romero, L.M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128: 1–24.

Roques, S., C. Diaz-Paniagua, and A. C. Andreu. 2004. Microsatellite markers reveal multiple paternity and sperm storage in the Mediterranean Spur-thighed Tortoise, *Testudo graeca*. Can. J. Zool. 82: 153–159.

Roques, S., C. Diaz-Paniagua, A. Portheault, N. Perez-Santigosa, and J. Hidalgo-Vila. 2006. Sperm storage and low incidence of mutliple paternity in the European Pond Turtle, *Emys orbicularis*: a secure but costly strategy? Biol. Conserv. 129: 236–243.

Rosen, P. C., and C. H. Lowe. 1994. Highway mortality of snakes in the Sonoran desert of Southern Arizona. Biol. Conserv. 68:143-148.

Rugiero, L., G. Milana, F. Petrozzi, M. Capula, and L. Luiselli. 2013. Climate-change-induced shifts in annual phenology of a temperate snake during the last 20 years. Acta Oecol. 51: 42–48.

Saint Girons, H. 1975. Sperm survival and transport in the female genital tract of reptiles. Pp. 105–113 *In* E. S. E. Hafez and C. G. Thibault (Eds.), The Biology of Spermaozoa. S Karger Publications, Basel, Switzerland.

Saint Girons, H. 1982. Reproductive cycles of male snakes and their relationships with climate and female reproductive cycles. Herpetologica 38: 5–16.

Sarvella, P. 1973. Adult parthenogenetic chickens. Nature 243: 171.

Schuett, G. W. 1992. Is long-term sperm storage an important compontent of the reproductive biology of temperate pitvipers? Pp. 169–184 *In* J. A. Campbell and E. D. Brodie Jr. (Eds.), Biology of the Pitvipers. Selva, Tyler, Texas.

Schuett, G. W., S. L. Carlisle, A. T. Holycross, J. K. O'Leile, D. L. Hardy Sr., E. A. Van Kirk, and W. J. Murdoch. 2002. Mating system of male Mojave Rattlesnakes (*Crotalus scutulatus*): seasonal timing of mating, agonistic behavior, spermatogenesis, sexual segment of the kidney, and plasma sex steroids. Pp. 515–532 *In* G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene (Eds.), Biology of the Vipers. Eagle Mountain Publishing, LC, Eagle Mountain, Utah.

Schuett, G. W., P. F. Fernandez, W. F. Gergits, N. J. Casna, D. Chiszar, H. M. Smith, J. B. Mitton, S. P. Mackessy, R. A. Odum, and M. J. Demlong. 1997. Production of offspring in the absence of males: evidence for facultative parthenogenesis in bisexual snakes. Herpetol. Nat. Hist. 5: 1–10.

Schuett, G. W., and J. C. Gillingham. 1986. Sperm storage and mutliple paternity in the Copperhead, *Agkistrodon contortrix*. Copeia 1986: 807–811.

Schuett, G. W., M. S. Grober, E. A. Van Kirk, and W. J. Murdoch. 2004. Long-term sperm storage and plasma steroid profile of pregnancy in a Western Diamond-backed Rattlesnake (*Crotalus atrox*). Herpetol. Rev. 35: 328–333.

Schuett, G. W., D. L. Hardy Sr., H. W. Greene, R. L. Earley, M. S. Grober, E. A. Van Kirk, and W. J. Murdoch. 2005. Sympatric rattlesnakes with contrasting mating systems show differences in seasonal patterns of plasma sex steroids. Anim. Behav. 70: 257–266.

Schuett, G. W., R. A. Repp, E. N. Taylor, D. F. DeNardo, R. L. Earley, E. A. Van Kirk, and W. J. Murdoch. 2006. Winter profile of plasma sex steroid levels in free-living male Western Diamond-backed Rattlesnakes, *Crotalus atrox* (Serpentes: Viperidae). Gen. Comp. Endocrinol. 149: 72–80.

Schuett, G. W., R. A. Repp, and S. K. Hoss. 2011. Frequency of reproduction in female Western Diamond-backed Rattlesnakes from the Sonoran Desert of Arizona is variable in individuals: potential role of rainfall and prey densities. J. Zool. 284: 105–113.

Schuett, G. W., R. A. Repp, M. Amarello, and C. F. Smith. 2013a. Unlike most vipers, female rattlesnakes (*Crotalus atrox*) continue to hunt and feed throughout pregnancy. J. Zool. 289: 101–110.

Schuett, G. W., R. A. Repp, S. K. Hoss, and H.-W. Herrmann. 2013b. Environmentally cued parturition in a desert rattlesnake, *Crotalus atrox*. Biol. J. Linn. Soc. 110: 866–877.

Schuster, S. M., and M. J. Wade. 2003. Mating Systems and Strategies. Princeton University Press, Princeton, New Jersey.

Schwartz, J. M., G. F. McCracken, and G. M. Burghardt. 1989. Multiple paternity in wild populations of the Garter Snake, *Thamnophis sirtalis*. Behav. Ecol. Sociobiol. 25: 269–273.

Seigel, R. A., H. S. Fitch, and N. B. Ford. 1986. Variation in relative clutch mass in snakes among and within species. Herpetologica 42: 179–185.

Sever, D. M., and R. Brizzi. 1998. Comparative biology of sperm storage in female salamanders. J. Exp. Zool. 282: 460–476.

Sever, D. M., and W. C. Hamlett. 2002. Female sperm storage in reptiles. J. Exp. Zool. 292:187–199.

Sever. D. M., and T. J. Ryan. 1999. Ultrastructure of the reproductive system of the Black Swamp Snake (*Seminatrix pygaea*): Part 1. Evidence for oviductal storage. J. Morphol. 241: 1–18.

Sever, D. M., J. L. Rheubert, J. Gautreaux, T. G. Hill, and L. R. Freeborn. 2012. Observations on the sexual segment of the kidney of snakes with emphasis on ultrastructure in the Yellow-bellied Sea Snake, *Pelamis platurus*. Anat. Rec. 295: 872–885.

Shields, W. M. 1987. Dispersal and mating systems: investigating their causal connections. Pp. 3–24 *In* B. D. Chepko-Sade and Z. T. Halpin (Eds.), Mammalian Dispersal Patterns: The Effects of Social Structure on Population Genetics. University of Chicago Press, Chicago, Illinois.

Shine, R. 1988. Parental care in reptiles. Pp. 275–330 *In* C. Gans and R. B. Huey (Eds.), Biology of the Reptilia, vol. 16. Alan R. Liss Inc, New York, New York.

Shine, R., M. M. Olsson, and R. T. Mason. 2000. Chastity belts in Garter Snakes: the functional significance of mating plugs. Biol. J. Linn. Soc. 70: 377–390.

Siegel, D. S., and D. M. Sever. 2006. Utero-muscular twisting and sperm storage in viperids. Herpetol. Conserv. Biol. 1: 87–92.

Siegel, D. S., and D. M. Sever. 2008. Sperm aggregations in female *Agkistrodon piscivorus* (Reptilia: Squamata): a histological and ultrastructural investigation. J. Morphol. 269: 189–206.

Simonov, E., and M. Wink. 2011. Cross-amplification of microsatellite loci reveals multiple paternity in Halys Pit Viper (*Gloydius halys*). Acta Herpetol. 6: 289–295.

Sinclair, E. A., J. B. Pramuk, R. L. Bezy, K. A. Crandall, and J. W. Sites Jr. 2010. DNA evidence for nonhybrid origins of parthenogenesis in natural popuations of vertebrates. Evolution 64: 1,346–1,357.

Sinervo, B., F. Méndez-de-la-Cruz, D. B. Miles, B. Heulin, E. Bastiaans, M. Villagrán-Santa Cruz, R. Lara-Resendiz, N. Martínez-Méndez, M. L. Calderón-Espinosa, R. N. Meza-Lázaro, H. Gadsden, L. J. Avila, M. Morando, I. J. De la Riva, P. V. Sepulveda, C. F. D. Rocha, N. Ibargüengoytía, C. A. Puntriano, M. Massot, V. Lepetz, T. A. Oksanen, D. G. Chapple, A. M. Bauer, W. R. Branch, J. Clobert, and J. W. Site Jr. 2010. Erosion of lizard diversity by climate change and altered thermal niches. Science 328: 894–899.

Smith, C. F., and G. W. Schuett. 2015. Putative pair-bonding in Copperheads (*Agkistrodon contortrix*) during the breeding season. Northeast. Nat. 22: N1-N5.

Smith, C. F., G. W. Schuett, R. L. Earley, and K. Schwenk. 2009. The spatial and reproductive ecology of the Copperhead (*Agkistrodon contortrix*) at the northeastern extreme of its range. Herpetol. Monogr. 23: 45–73.

Smith, C. F., G. W. Schuett, and K. Schwenk. 2010. Relationship of plasma sex steroids to the mating season of Copperheads at the north-eastern extreme of their range. J. Zool. 280: 362–370.

Smith, C. F., G. W. Schuett, and S. K. Hoss. 2012. Reproduction in female Copperhead Snakes (*Agkistrodon contortrix*): plasma steroid profiles during gestation and post-birth periods. Zool. Sci. 29: 273–279.

Smith, C. F., G. W. Schuett, and M. Amarello. 2015. Male mating success in a North American pitviper: role of body size, testosterone, and spatial metrics. Biol. J. Linn. Soc. 115: 185–194.

Smith, L. B., and W. H. Walker. 2014. The regulation of spermatogenesis by androgens. Semin. Cell Dev. Biol. 30: 2–13.

Sparkman, A. M., S. J. Arnold, and A. M. Bronikowski. 2007. An empirical test of evolutionary theories for reproductive senescence and reproductive effort in the Garter Snake *Thamnophis elegans*. Proc. R. Soc. B. Biol. Sci. 274: 943–950.

Staub, N. L., and M. De Beer. 1997. The role of androgens in female vertebrates. Gen. Comp. Endocrinol. 108: 1–24.

Sugg, D. W., and R. K. Chesser. 1994. Effective population sizes with multiple paternity. Genetics 137: 1,147–1,155.

Taylor, E. N., and D. F. DeNardo. 2005. Sexual size dimorphism and growth plasticity in snakes: an experiment on the Western Diamond-backed Rattlesnake (*Crotalus atrox*). J. Exp. Zool. A. 303: 598–607.

Taylor, E. N., and D. F. DeNardo. 2010. Hormones and reproductive cycles in snakes. Pp. 355–372 *In* D. O. Norris and K. H. Lopez (Eds.), Hormones and Reproduction of Vertebrates, Vol. 3. Academic Press, San Diego, California

Taylor, E. N., D. F. DeNardo, and D. H. Jennings. 2004. Seasonal steroid hormone levels and their relation to reproduction in the Western Diamond-backed Rattlesnake, *Crotalus atrox* (Serpentes: Viperidae). Gen. Comp. Endocrinol. 136: 328–337.

Taylor, E. N., M. A. Malawy, D. M. Browning, S. V. Lemar, and D. F. DeNardo. 2005. Effects of food supplementation on the physiological ecology of female Western Diamond-backed Rattlesnakes (*Crotalus atrox*). Oecologia 144: 206–213.

Telemeco, R. S., K. C. Abbott, and F. J. Janzen. 2013. Modeling the effects of climate change-induced shifts in reproductive phenology on temperature-dependent traits. Am. Nat. 181: 637-648.

Tinkle, D. W. 1957. Ecology, maturation, and reproduction of *Thamnophis sauritus proximus*. Ecology 38: 69–77.

Tokarska, M., T. Marshall, R. Kowalczyk, J. M. Wójcik, C. Pertoldi, T. N. Kristensen, V. Loeschcke, V. R. Gregersen, and C. Bendixen. 2009. Effectiveness of microsatellite and SNP markers for parentage and identity in species with low genetic diversity: the case of European Bison. Heredity 103: 326–332.

Uller, T., and M. Olsson. 2008. Multiple paternity in reptiles: patterns and processes. Mol. Ecol. 17: 2,566–2,580.

United Nations. 2007. Population urbanization prospects: The 2007 revision. (http://www.un.org/esa/population/meetings/EGM_PopDist/Heilig.pdf: viewed 16 November 2014).

Ursenbacher, S., C. Erny, and L. Fumagalli. 2009. Male reproductive success and multiple paternity in wild, low-density populations of the Adder (*Vipera berus*). J. Hered. 100: 365–370.

Vaughan, M. S., and R. A. Steele. 2014. Natural History Notes. *Bothrops asper* (Terciopelo). Parthenogenetic reproduction. Herpetol. Rev. 45: 705.

Vicosa, B., J. J. Emerson, Y. Zekster, S. Mahajan, and D. Bachtrog. 2013. Comparative sex chromosome genomics in snakes: differentiation, evolutionary strate, and lack of global dosage compensation. PLoS Biol. 11: e1001643.

Walther, G-R., E. Post, P. Convey, A. Menzel, C. Parmesank, T. J. C. Beebee, J.-M. Fromentin, O. Hoegh-GuldbergI, and F. Bairlein. 2002. Ecological responses to recent climate change. Nature 416: 389–395.

Wapstra, E., T. Uller, D. L. Sinn, M. Olsson, K. Mazurek, J. Joss, and R. Shine. 2009. Climate effects on offspring sex ratio in a viviparous lizard. J. Anim. Ecol. 78: 84–90.

Weatherhead, P. J., M. R. Prosser, H. L. Gibbs, and G. P. Brown. 2002. Male reproductive success and sexual selection in Northern Water Snakes determined by microsatellite DNA analysis. Behav. Ecol. 13: 808–815.

Weinman, L. R., J. W. Solomon, and D. R. Rubenstein. 2015. A comparison of single nucleotide polymorphism and microsatellite markers for analysis of parentage and kinship in a cooperatively breeding bird. Mol. Ecol. Resour. 15: 502–511.

Winne, C. T., J. D. Willson, B. D. Todd, K. M. Andrews, and J. W. Gibbons. 2007. Enigmatic decline of a protected population of Eastern Kingsnakes, *Lampropeltis getula*, in South Carolina. Copeia 2007: 507–519.

Wynn, A. H., C. J. Cole, and A. Gardner. 1987. Apparent triploidy in the unisexual Brahminy Blind Snake, *Ramphlotyphlops braminus*. Am. Mus. Novit. 2868: 1–7.

Zimmerman, E. G., and W. Kilpatrick. 1973. Karyology of North American crotaline snakes (Family Viperidae) of the genus *Agkistrodon*, *Sistrurus*, and *Crotalus*. Canad. J. Genet. Cytol. 15: 389–395.