What explains genital variation in snakes? A review of the hypotheses of genital co-evolution

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Abstract

Genitalia can be defining traits for many species with internal fertilization. In general, genitalia of males and females typically coevolve, as copulation is one of the most mechanically direct interactions in biology. Snakes, in particular, have quite complex and elaborate genitalia. Male snakes possess paired copulatory organs, known as hemipenes. There is immense variation in these structures, including spines, scoops, size, and the presence of bilobes. Females accordingly have complementary variation in genital structures. Three hypotheses exist to explain this variation and coevolution: the lock-and-key hypothesis, sexual conflict, and cryptic female choice. In an attempt to identify the main driver of this variation and coevolution, this review will investigate each of these hypotheses to explain genitalia variance, and it will evaluate the primary evidence behind each of them. Present evidence majorly supports the sexual conflict hypothesis and the lock-and-key hypothesis. Ultimately, however, further research on female snake genitalia, and on snake genitalia in general, should be completed, as there are still many unanswered questions.

Introduction

In many animals with external genitalia and internal fertilization, the genitals are often elaborate and unique. Males typically have intromittent copulatory organs, and females have an internal genital tract which receives the male intromittent copulatory organ during copulation. Traditionally, male genitalia have been considered more diverse and variable, compared to female genitalia (Eberhard 1985). Female genitalia have not been considered as variable due to lack of research, primarily due to male researcher bias and the hidden nature of female genitalia (Ah-King et al. 2014; Brennan & Prum 2015). Furthermore, female genitalia can be complicated to study, as they are subject to other selective pressures. The female reproductive tract not only receives the male intromittent copulatory organ during copulation, but it also performs birthing functions, sperm storage, and ovipositing (Brennan & Prum 2015). Despite these multiple functions, female genitalia are still considered variable enough for coevolution to occur (Brennan 2016; Brennan & Prum 2015). When discussing genital coevolution, it is vital to have variation in both the male and the female genitalia.

Genital coevolution occurs when evolutionary changes in one sex's genitalia drives evolutionary change in the opposite sex's genitalia (Brennan & Prum 2015). As copulation is one of the most mechanically specific interactions between individuals, genitalia of the male and female must evolve closely together in order for copulation to be successful (Brennan 2016; Brennan & Prum 2015). Initially, Charles Darwin proposed that natural selection influences genital coevolution, as copulation is a function of gamete transfer and is necessary for successful reproduction (Darwin 1871). In addition to natural selection, genital coevolution can occur through other mechanisms, such as sexual selection. Experimental evolution has confirmed that genital coevolution occurs under sexual selection pressures and through mate choice (Simmons & Garcia-Gonzalez 2011). Nonetheless, there are three hypotheses that are frequently used to explain the roots of genital coevolution that incorporate both natural and sexual selection: lock-and-key, sexual conflict, and cryptic female choice.

The lock-and-key hypothesis is the most popularly known hypothesis regarding genital coevolution (Eberhard 2010). This hypothesis proposes that genital coevolution occurs through natural selection in order to prevent hybridization and to reinforce reproductive isolation (Dufour 1844; Eberhard 2010; Shapiro & Porter 1989). This is advantageous because hybridizations can be quite costly, especially for females, as they lose a large investment if they produce an inviable or unfit offspring from mating with a heterospecific male (Eberhard 2010). It can also be costly for males, although to a lesser extent, as they do not typically invest as much in the offspring as females do (Bateman 1948). Thus, the genitalia coevolve to only fit with the lock or key of their own species (Brennan &

Prum 2015). Under this hypothesis, the genitalia are expected to have a close mechanical fit during copulation and specific coevolution of genital shape (Brennan & Prum 2015). It would also be expected that this would occur when closely related species are living in sympatry, as this is when the risk of hybridization would be the highest. The lock-and-key hypothesis tends to be refuted, however, as female genitalia are not viewed as variable enough to possess species-specific locks (Eberhard 2010). This claim is largely based on the fact that female genitalia are largely understudied, due to male researcher bias and the hidden nature of female genitalia (Ah-King et al. 2014). However, there is now evidence that demonstrates the falsity in this claim, and that female genitalia are in fact also variable (Simmons 2014). With variation in both male and female genitalia, there is a good foundation for supporting the lock-and-key hypothesis.

Another hypothesis that can explain genital coevolution is sexual conflict, which results in sexually antagonistic coevolution, initiating a coevolutionary arms race. Under this hypothesis, males and females compete for control over reproduction, but the genital adaptations that benefit each sex are detrimental to the opposite sex (Chapman et al. 2002; Eberhard 2010). Sexual conflict may occur through male-male competition for a successful fertilization or through natural selection on female behavior, physiology, or morphology, allowing the female to reduce physical harm or resist coercion (Brennan & Prum 2015). One of the best-known examples of sexually antagonistic coevolution is in waterfowl, where males are forceful in their mating attempts with females; this comes at a disadvantage to the female (Brennan et al. 2007). Males have a corkscrew shaped penis and females have a vagina shaped in a way to prevent coercive mating attempts from the males (Brennan et al. 2007). Here, the genitalia of the male and female waterfowl oppose each other, as they fight to gain control over copulation. It can be expected under this hypothesis that the female genitalia or body would be harmed, but that is not always the case when genitals coevolve through sexual selection.

The final hypothesis that can explain genital coevolution is cryptic female choice (CFC), which operates through sexual selection. Under this hypothesis, genital coevolution occurs because females prefer certain sensory stimuli from male genitalia to facilitate a mate choice or become pregnant (Brennan & Prum 2015). Some features of the male genitalia will be more stimulating, and thus will be selected for (Eberhard 2010). In domestic pigs (Sus domesticus), the females require stimulation from a filament on the male penis in order to become pregnant; if they do not receive this stimulation, such as in artificial insemination, pregnancy rates drop (Bonet et al. 2013). In this case, the female need for stimulation drives the evolution of penis shape. This hypothesis also requires female promiscuity, as females must be choosing from multiple males (Brennan & Prum 2015). Additionally, under this hypothesis, male genitalia should not harm the females, as it may under the sexual conflict hypothesis (Friesen et al. 2014). All these three hypotheses mentioned—lock-and-key, sexual conflict, and cryptic female choice—can be investigated further in snakes—a good model for studying genitalia.

In snakes (class Reptilia; order Squamata), the genitalia are elaborate and unique. Male snakes possess paired intromittent copulatory organs, termed hemipenes, and females accordingly have hemiclitores, as well as vaginal pouches (Gredler et al. 2014). Hemipenes extend from the lateral edges of the cloacal opening when everted (Leal & Cohn 2015). These paired copulatory organs are present in lizards as well, which are also part of the Reptilian order, Squamata. Interestingly, unpaired intromittent copulatory organs are present in two other Reptilian orders, Testudines and Crocodylia, but not the order which contains the tuatara, Rhynchocephalia (Gredler et al. 2014). During embryonic development, hemipenes arise from lateral swellings on each side of the cloaca, and as the embryo develops, these swellings grow to form the right and left hemipenes (Leal & Cohn 2015). These organs undergo further development and differentiation as they transform into their mature form.

Once sexually mature, both male and female snakes have immense genital variation, both in physical structure and appearance. Male snake hemipenes have been photographically documented to have spines, scoops, and bilobes, or to have no complex structures at all (Andonov et al. 2017). Similarly, female genitalia, although still widely understudied in snakes, have some documented variation as well. Female pouch morphologies and vaginal shapes differ among species (Showalter et al. 2014; Siegel et al. 2012). The variation has been documented across many snake species and suggests a close genital coevolution. However, it is still unknown what explains this genital variation and coevolution,

and what the main force of evolution is behind it. In an attempt to locate the main driver of this genital variation and coevolution in snakes, this review will investigate each of the hypotheses that explain genital variance and coevolution, and it will evaluate the primary evidence behind each of them. This review will begin with the discussion of evidence behind the classic hypothesis of genital coevolution, the lock-and-key hypothesis. It will then transition into the two other hypotheses, sexual conflict and cryptic female choice, and highlight the evidence behind each of them.

Lock-and-key

The lock-and-key hypothesis states that genitalia of males and females evolved to prevent a costly hybridization event with another closely related species and also to reinforce reproductive isolation (Shapiro & Porter 1989). Under this hypothesis, a close mechanical fit between the genitalia is expected (Brennan & Prum 2015). In snakes, the evidence for the lock-and-key hypothesis of genitalia coevolution is limited, but still substantial.

Most broadly speaking, in male snakes, hemipenes are extremely variable, in regard to their shape, presence of spines, width, and length (Andonov et al. 2017). In simplest terms, if male hemipenes present such variation, it would make sense for the female genitalia to also be variable, in order to fit with these hemipenes. Accordingly, female snake genitalia have shown to be diverse. In female snakes, a "pouch" is present in the reproductive tract, and it is involved with the receiving of the male copulatory organ (Siegel et al. 2012). Siegel and colleagues (2012) found these pouches to be variable interspecifically in Colubridae snakes. This variation was substantial, as some pouches were bifurcated, separated, or classified as "simple." According to the lock-and-key hypothesis, variation should exist interspecifically, as to prevent costly hybridization events (Shapiro & Porter 1989). Because this variation was observed interspecifically, this evidence supports the lock-and-key hypothesis. This study, however, did not investigate if these species were living in allopatry or sympatry; only if they were living in sympatry would this be substantial evidence for lock-and-key, as closely related sympatric species have the highest rates of hybridization.

Further evidence supports genital variation in female snakes, which is suggestive of coevolution. Showalter and colleagues (2014) investigated interspecific genital variation in two closely related species of watersnakes (*Nerodia sipedon* and *Nerodia fasciata*) and discovered that the vagina is different in these two species living in sympatry. The vaginas were both bifurcated, but they differed enough in the degree of bifurcation. As these species are closely related and living in sympatry, this evidence greatly supports the lock-and-key hypothesis. Because these two species are already somewhat genetically similar and living in sympatry, further reinforcement of reproductive isolation is necessary to prevent a costly hybridization event (Brennan & Prum 2015). Genitalia specific to each species represents that reinforcement. Further supporting this hypothesis is evidence from King and colleagues (2009) which demonstrates that the male genitalia in these two species were morphologically similar; they were bilobed in accordance with the bifurcated female vagina (King et al. 2009). As expected under the lock-and-key hypothesis, the male and female genitalia in these two watersnake species demonstrate a mechanically close fit, also known as copulatory adjustment (Edgren et al. 1953), thus supporting that this hypothesis explains the variation present in these two species. Although there is substantial evidence supporting the lock-and-key hypothesis, there is also some strong evidence against it. Inger and Marx, two herpetologists from the Field Museum of Natural History in Chicago, Illinois, found that the genitalia of *Calamaria lumbricoidea* (family Colubridae) varied not interspecifically as expected under the lock-and-key hypothesis, but intraspecifically (Inger & Marx 1962). Interestingly, this variation was observed in populations not living in sympatry. This could indicate that a reproductive isolation event occurred, possibly leading to the beginnings of speciation. However, the female genitalia from one population fit well enough with the male genitalia of the other population; the same was true for the reverse. This refutes the lock-and-key hypothesis, as this example does not demonstrate a mechanically close fit, or copulatory adjustment. This instead supports that sexual selection via female choice is driving this variation, as females within the species may be selecting for certain male genitalia, driving intraspecific variance (Gilligan & Wensel 2008). All these stated examples denote that the lock-and-key hypothesis may explain variation in some species, but not in others, hinting that there must be other explanations for this variation and coevolution.

Sexual Conflict

Sexual conflict can result in sexually antagonistic coevolution, initiating a coevolutionary arms race in an effort to gain control over reproduction. In this instance, genitalia can coevolve to benefit the one sex, but harm the other. In the case of genital coevolution via sexual conflict, genitals can be expected to evolve very fast. In male Anolis lizards, their genitalia have been observed to evolve faster than any other trait they possess (Klaczko et al. 2015). Like snakes, lizards are in the order Squamata, and they possess hemipenes. This example, although not in snakes, may be surface level evidence of sexual conflict driving genital coevolution, as coevolutionary arms races are characterized by rapid genitalia evolution (Hosken & Stockley 2004). The sexual conflict hypothesis of genital coevolution can be further investigated by looking at male and female genital adaptations, as well as adaptive behaviors in each sex that respond to the reproductive conflict.

Male genital adaptations

Male hemipenes have a wide variation in traits that are characteristic of conflict, such as the presence of spines, scoops, and bilobes (Andonov et al. 2017; Friesen et al., 2014; King et al. 2009). Although these characters are not inherently linked to conflict—they have the possibility of stimulating the female in a way that signals male quality—there is substantial evidence claiming that they are working against the females, leading to conflict. There is also evidence that they are not simply a vestigial structure, as once thought, relating to the loss of limbs in snakes. Nunes and colleagues (2014) presented evidence that limb reduction is not related to the presence of hemipenile spines in lizards that have limb loss; it can be inferred that the same applies for snakes, as snakes and lizards are closely related. As this idea has not been supported, it seems even more probable that hemipenile spines are involved in sexual conflict.

In support of spines leading to sexual conflict, Friesen and colleagues (2014) observed copulatory behavior in red-sided garter snakes (*Thamnophis sirtalis*) and found that spines function to extend copulation by holding the female in place; spines allow the male to secure the copulatory organs together. When spines were removed, copulation was observed to be much shorter, as females could then end copulation when they wished (Friesen et al. 2014). Interestingly, females were also bleeding during or after copulation. Together, these results indicate that hemipenile spines are not only an adaptation to extend copulation past the point of the female's preference, but they are also physically damaging to the female—a characteristic of sexual conflict. Both these findings support that male snake genitalia possesses an adaptation that leads to conflict between the sexes. In other animals as well, penile spines have been studied in relation to the harm they cause females. Penile spines in male seed beetles damage females after just one copulation event (Ronn et al. 2007). Interestingly, female seed beetles can adapt to these spines; this study demonstrated that as penile spines increased, the thickness of the reproductive tract wall in females also increased. As both the males and the females are evolving adaptations in response to the genitalia of the opposite sex, this demonstrates that a sexually antagonistic arms race is occurring, indicative of sexual conflict. Although studies have not investigated the relationship between increased hemipenile spines and the thickness of the reproductive tract wall in female snakes, this relationship is highly probable, as hemipenile spines are clearly damaging to females (Friesen et al. 2014). Female snakes could be expected to adapt in a way that protects them from these spines, as the female seed beetles do. Another variation in the shape of the male hemipenis in snakes is the scoop. This scoop shape is present in the Eastern Montpellier snake (*Malpolon insignitus*), and it is quite prominent (Andonov et al. 2017). To date, there is no explanation for this shape, however, evidence in other non-snake species may point to a possible function for this scoop shape. In damselflies, the scooped shaped tip of the penis functions in removing the sperm of other males from inside the female reproductive tract (Waage 1979). Many female snakes can store sperm (Friesen et al. 2013a) and mate with multiple males, so this may explain the scoop shape in the hemipenes of *Malpolon insignitus* (Andonov et al. 2017). If true, this male genital characteristic would support the sexual conflict hypothesis as an explanation of variation and coevolution in snake genitalia. Further, another hemipenile characteristic that may explain the coevolution of genitalia via sexual conflict is the presence of bilobes. King and colleagues (2009) observed longer copulations in Plains garter snakes (*Thamnophis radix*) with bilobed hemipenes; this was paired with female body rolling behavior and resistance to the copulation (King et al. 2009). This demonstrates that a characteristic of the male genitalia is causing conflict, as the females did not want to engage in copulation that long. The presence of bilobes enables males to gain control over copulation in a way that directly benefits them. Moreover, as time goes

on, female snakes in species with males possessing bilobed hemipenes may evolve resistance to this trait through a physical genital adaptation. Aside from hemipenile characteristics, males have other genital adaptations, such as the ability to insert a mating plug into females, which is observed in garter snakes (*Thamnophis sirtalis*) (Friesen et al. 2013b). Sperm plugs enable the sperm to be inserted inside the female and gradually released, improving the mating success rate. It may also prevent sperm from another male from fertilizing the female (Mangels et al. 2016). This ability represents a male genital adaptation to override the female behavioral adaptation to end copulation early and prevent the male from depositing enough sperm; in this instance, the male and female adaptations are in conflict with each other, demonstrating sexual conflict. Additional studies have also demonstrated this behavior in female snakes, indicating that sperm plug deposition may be common (King et al. 2009; Shine et al. 2003). With these examples, it is important to note that behaviors that exist to prevent or sustain copulation can be included in the definition of genital coevolution, as they too can influence the evolution of genital structures (Brennan, 2016). Behaviors can be just as important as genital structure in driving genital coevolution.

Female genital adaptations

Female snakes also have genital adaptations that work against the male genitalia, leading to sexual conflict. Friesen and colleagues (2014) found that after anesthetizing the female pouch in red-sided garter snakes (*Thamnophis sirtalis*), copulation was much longer, ending when the male ended it. It is important to note that the male also possessed hemipenile spines to support a long copulation (Friesen et al. 2014). Through this experiment, they discovered that female pouches are muscularized, allowing the female to contract the pouch and expel the male hemipenis prematurely. This demonstrates that the female pouch, which is shown to have large interspecific variation (Siegel et al. 2012), is an adaptation to gain control over copulation and counter male genital adaptations, such as hemipenile spines or sperm plugs. Thus, the female pouch is a genital characteristic that opposes male genital adaptations, supporting the sexual conflict hypothesis for genital coevolution.

Female behavioral adaptations

Similarly, as briefly mentioned prior, female snakes also have behavioral adaptations that provide evidence of sexual conflict driving genital coevolution. In several different snake species, female behaviors to end copulation early have been observed, which supports the idea that male snake genitalia are causing sexual conflict. Females have been observed body rolling to terminate copulation (King et al. 2009), and they have also been observed displaying behavior to directly counter forcible insemination (Shine et al. 2003). Shine and colleagues (2003) demonstrated that male garter snakes (*Thamnophis sirtalis*) exploit the physiology of the female snakes, initiating hypoxic stress which causes them to raise their tail and gape their cloaca. Although these behaviors are not directly related to genitalia, they provide an opportunity for genital adaptations to evolve in the future, in response to these behavioral traits. Female snakes may acquire a genital adaptation that allows them to gain control over copulation in response to this forcible insemination.

Cryptic Female Choice

Cryptic female choice is the final hypothesis proposed to explain causes of genital coevolution. This hypothesis suggests that females select for certain male genitalia that stimulates them in a way that indicates mate quality (Brennan & Prum 2015; Eberhard 2010). Generally, the evidence for cryptic female choice driving genital coevolution in snakes is quite weak. Traditionally, spines have been thought of as a possible stimulator, allowing females to select for certain males that provide that stimulation; however, this idea has been refuted. As discussed earlier under the sexual conflict hypothesis, spines cause damage to the female. Female red-sided garter snakes were observed bleeding during and after copulating with a male with spined hemipenes (Friesen et al. 2014). Additionally, in seed beetles, hemipenile spines are also damaging, showing damage to the female reproductive tract after just one instance of mating (Ronn et al. 2007). Under cryptic female choice, a male trait that stimulates the female in a way that enables her to exhibit choice will not cause damage.

Further, weak evidence also presents a possible instance of female choice occurring in snakes. Friesen et al. (2013) demonstrated that red-sided garter snake females do not appear to be inherently receptive to sperm, as when males were prevented from depositing mating plugs, sperm leaked out of the female cloaca. This seems disadvantageous, but it may be a female adaptation for exhibiting choice. If females do not want sperm from

a certain male, they may allow it to leak out, instead of contracting it up into the reproductive tract. However, it is unknown if in a different case, perhaps with a high genetic quality male, the outcome would be different. To test this, a quality male and a low-quality male would need to be bred with a female to observe her behavior of sperm uptake and leakage. If the female allowed less sperm from the quality male to leak, the evidence would provide support for a female genital adaptation driving genital coevolution. Moreover, as mentioned prior, the intraspecific variation observed in *Calamaria lumbricoidea* by Inger & Marx (1962) may be due to female choice. Intraspecific variation in genitalia suggests that sexual selection is occurring via female choice, specifically, cryptic female choice (Gilligan & John 2006). In intraspecific populations, males vary in quality and females choose among them, driving variance in primary and secondary sex characteristics, such as genitalia. Thus, it is quite possible that this intraspecific variation in *C. lumbricoidea* is due to female choice for a specific genital characteristic. Further research would be beneficial to confirm if this species needs stimulation in order to become pregnant, as seen in domestic pigs (Bonet et al. 2013). If stimulation is needed in snakes, that would be substantial evidence for this hypothesis.

Conclusion

As addressed in this review, significant and convincing evidence exists to provide reasoning behind genital coevolution and corresponding variation in snakes. The sexual conflict and lock-and-key hypotheses appear to have the most substantial evidence behind them, with cryptic female choice having the least support; however, it appears that each unique species and situation has a different hypothesis that strongly supports it. Certain species have very convincing evidence for one hypothesis, but others present evidence that refute that same hypothesis. There may not be one answer for all snakes that explains genital coevolution and corresponding variance. We should, however, before coming to a definite conclusion, investigate female genitalia in greater depth, as many unanswered questions lie in the female genitalia. Nonetheless, this review highlights important areas of biology, and sheds light on how studying genitalia may be useful in other areas of ecology, conservation biology, as well as evolutionary biology. Studying genitalia has implications in defining species, which serves purposes in conservation biology. Determining if species are reproductively isolated or not may yield them a better chance at conservation, as the Endangered Species Act employs the Biological Species Concept, a concept which defines a species based on reproductive isolation (Wheeler & Meier 2000). Additionally, studying snake genitalia in particular may shed light on the evolutionary origins of the hemipenis, and help gain a better understanding of the vastness of reptile genitalia in general. Most importantly, future studies are needed to investigate female snake genitalia in greater depth, as it is overall still widely understudied (Ah-King et al. 2014). Studying female genitalia will allow further confirmation of these three hypotheses, either for the whole group of snakes, or for individual species or families of snakes. Furthermore, future studies should be completed on the genitalia of snake species living in sympatry, as further evidence of interspecific variation in sympatric species would help support the lock-and-key hypothesis.