

Effect of Male Presence on Reproductive Activity in Captive Female Blood Pythons, *Python curtus*

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Proper timing of the reproductive cycle to assure mate access is critical to optimizing success. We tested the importance of male presence on the progression of the female reproductive cycle in the blood python, *Python curtus*. Upon reaching sexual maturity, captive-born, individually raised virgin female snakes were housed with either a conspecific male or female. At the onset of the reproductive season (late autumn), all females initiated early follicular growth; however, only females housed with males initiated vitellogenesis, and these females ovulated and oviposited viable eggs. Females not housed with males showed arrested follicular development in that they maintained hydrated, nonvitellogenic follicles that underwent follicular regression after three months. The requirement for male presence prior to vitellogenesis is premature for fertilization but assures the female of a mate prior to mobilizing substantial energy stores into reproduction. Once an energy investment is made, females commit to completing the cycle.

SUCCESSFUL reproduction is highly dependent on the timing of reproductive events. Mates, or minimally their sperm, must be available at the appropriate stage of a female's reproductive cycle to maximize fertilization, and offspring should be produced at a time that optimizes their survival. Proximate environmental factors provide cues that initiate the breeding season and orchestrate the sequence of events once the reproductive cycle is initiated (Wingfield and Moore, 1987). These factors can be classified into four major groups: (1) "initial predictive information," which provides the stimulus for the onset of reproduction; (2) "supplementary information," which fine tunes the initial information to conform more closely to the local environment and the condition of the given year; (3) "synchronizing and integrating information," which coordinate the reproductive effort of individual animals; and (4) "modifying factors," which can interrupt breeding (Wingfield, 1983).

In snakes, change in environmental temperature is the most common initial predictive factor that stimulates the onset of reproductive activity (Bona-Gallo and Licht, 1983; Whittier et al., 1987). A delineated reproductive season assures that eggs are laid and offspring born when environmental conditions are optimal for survival. In the reproductive season, it is critical that reproduction is synchronized at the individual level to assure that a mate is available. Most species of snake are solitary, and females are not continually in the presence of males. Therefore, unlike the case in social species, access to a male during critical periods in the reproductive cycle is not necessarily guaranteed.

Female snakes enhance the probability of having sperm available to fertilize ova at the critical period by secreting pheromones to attract males (for review, see Mason, 1992) and by retaining sperm for future fertilizations (for review, see Schuett, 1992). Although these mechanisms increase the likelihood of successful reproduction, neither directly regulates the female reproductive cycle. Without a direct regulator, females could make large investments into vitellogenesis even if a male is not available.

In some mammals (e.g., cats, ferrets, rabbits), the female reproductive cycle is directly regulated in that ovulation is triggered by neuroendocrine stimulation derived from copulation (Chaffaux, 1993). In the red-sided garter snake, *Thamnophis sirtalis parietalis*, copulation plays a critical role in synchronizing reproduction by facilitating vitellogenesis (Bona-Gallo and Licht, 1983; Garstka et al., 1985; Mendonca and Crews, 1990). In this species, however, there appear to be additional cues that can override the necessity of mating for oviducal recrudescence (Whittier and Crews, 1986). Whether the stimulatory effect of copulation on vitellogenesis is unique to *Thamnophis sirtalis* or a general phenomenon of snakes remains unknown. The goal of this study was to determine the importance of male presence on vitellogenesis in a species of boid snake, *Python curtus*. We hypothesize that vitellogenesis in this species is stimulated by male presence and that females lacking males do not undergo vitellogenesis.

MATERIALS AND METHODS

Subjects for this study were a group of 18 (6 males, 12 females) *P. curtus*, born in 1988. Ani-

imals were individually reared in 37×28 cm plastic boxes with a strip of electrical heating tape underneath one end of the cages to provide a temperature gradient of 20–35 C. In August 1991, the subjects were paired into plastic cages with a floor space of 122 cm \times 61 cm (Neodesha, Neodesha, KN). The cages were opaque on all sides except the front. Each cage was equipped with a heating pad (with the thermostat at the “medium” setting) placed under one side of the cage, a water bowl large enough to enable a snake to soak, and a hide box. The snakes were offered food weekly, initially consisting of a diet of domestic mice, followed by domestic rats and small domestic rabbits as the subjects increased in size.

Females were randomly assigned to cages so that six cages housed one female and one male and three cages housed two females each. Females in the latter group, therefore, never had physical contact with a male since hatching. All cages were located against a single wall, preventing visual contact between snakes in different cages. This arrangement controlled for the number of animals per cage but did not allow us to distinguish results based on stimulatory effects of males versus inhibitory effects of females. There is, however, no evidence of female-female aggression in this species and no evidence of female-induced inhibition of reproduction in any species of snake. Therefore, given the limited sample size, we opted to control for the number of animals per cage.

Throughout the reproductive season (October 1991 to May 1992), we examined the reproductive condition of females approximately bi-weekly (i.e., 13 examinations over six months) using ultrasonography. Reproductive condition was recorded in terms of general reproductive state and average diameter of representative follicles or ova, and four reproductive states were assessed. Females with no measurable follicles were considered “inactive.” “Early follicular growth,” or the hydration phase (Dessauer and Fox, 1959), consists mainly of an increase in the water content of the follicle. “Vitellogenesis” and the associated deutoplasmic phase of follicular growth (Dessauer and Fox, 1959), is the period when calcium, lipids, proteins, and phosphoproteins are deposited in the developing follicles. Females were categorized into the final reproductive state following “ovulation.”

Reproductive states were determined by observing distinctive differences in ultrasonic images. If ovaries were not detectable or follicles not measurable during ultrasound imaging, the female was considered reproductively inactive. Ovaries in early follicular growth were identified

as round echolucent masses (6–20 mm diameter) grouped in either a chain or cluster. Vitellogenesis was determined by the presence of echogenic masses (20–30 mm diameter) grouped in clusters and limited to the area of the ovaries. Although vitellogenesis and yolk deposition can be uncoupled by using hormonal manipulation (Garstka et al., 1985), they are tightly linked in unmanipulated snakes. Therefore, the presence of deutoplasmic follicles is an accurate means of assessing vitellogenesis. After ovulation, females had round or oblong large masses (> 30 mm diameter) aligned in a row and occupying the majority of the distal coelomic cavity. We used an Ausonics Microimager 1000 ultrasound machine with a 5.0 megaHertz transducer. One female that was not exposed to a male became a reluctant feeder; thus, data from her were not included in the analysis.

We used chi-square analysis to assess the difference in reproductive state between the two groups. Additionally, we used Mann-Whitney *U*-tests to evaluate the difference between follicle/ovum size at each time point. Differences were considered significant where $P < 0.05$. We used the computer program StatView (SAS Institute, Inc., Cary, NC) for all analyses.

RESULTS

Exposure to males had a significant effect on the reproductive cycle of female *P. curtus* ($X^2 = 7.739$, $P = 0.01$). At the time of initial examination (29 October 1991), all females were determined to be reproductively inactive. On 25 November 1991, four females (two from each treatment group) had initiated early follicular growth, and by 23 December 1991, all females had reached this state. None of the females without males proceeded beyond this stage but, instead, maintained large hydrated follicles for approximately three months before they regressed (Fig. 1). Although courtship and mating were not observed, five of six females that were housed with males rapidly progressed through early follicular growth and vitellogenesis, proceeded through ovulation, and oviposited viable eggs (Fig. 1). The one female that was housed with a male but did not progress to vitellogenesis showed a follicular cycle similar to that of females not housed with males (i.e., maintaining hydrated follicles for a few months prior to follicular regression; Fig. 1).

Because the females without males were housed in pairs to control for the number of individuals per cage, the most conservative statistical approach is to treat pairs of females as a single observation. Therefore, we also calculat-

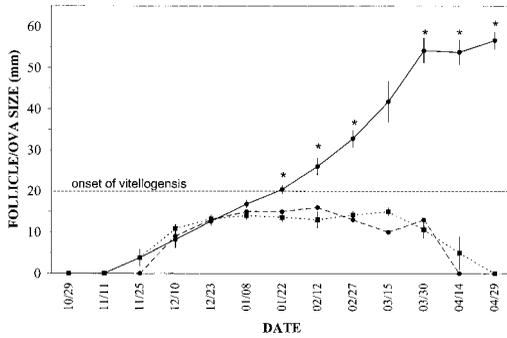


Fig. 1. Average follicle/ova size during the reproductive season for female *Python curtus*. Circles with the solid line represent the mean for females that were housed with a male and proceeded to oviposit a viable clutch ($n = 5$), circles with the dashed line represent the single female which was housed with a male but failed to proceed to vitellogenesis, and the squares with the dotted line represent females that were housed with another female ($n = 5$). Error bars represent ± 1 SE. Asterisks represent significant differences between all females housed with males and females housed without males (Mann-Whitney U -test, $P < 0.05$). Approximate follicle/ova size at the time of vitellogenesis is indicated on the graph.

ed the significance of the chi-square for $n = 3$. Even in this more conservative analysis, the effect of male presence was significant ($X^2 = 5.625$, $P = 0.02$).

DISCUSSION

We have demonstrated that male presence plays a significant role in regulating female reproduction in *P. curtus*. Females exposed to males rapidly progressed through their reproductive cycle, and those with no exposure to males initiated their cycle but terminated it prior to vitellogenesis. Although behavioral observations were not made, lack of such information does not negate the significance of these results. Requiring male presence and possibly copulation to initiate vitellogenesis assures a female of a mate prior to her investing substantial energy into reproduction, because female snakes can invest 30% or more of their body mass into a clutch (Greene, 1997).

Existing data on the regulation of the female reproductive cycle in snakes is limited and inconclusive. In *T. sirtalis*, the most extensively studied snake, the results are somewhat confounding. Mating appears to have an obligatory role in the onset of vitellogenesis (Bona-Gallo and Licht, 1983; Garstka et al., 1985; Mendonca and Crews, 1990) with the tactile stimulation of copulation acting as the regulating mechanism.

If this stimulation is blocked via spinal transection or the use of local anesthetics applied to the cloaca, the stimulatory effects of mating are inhibited (Mendonca and Crews, 1990). However, in most studies of *T. sirtalis*, rare individuals have been reported to proceed through vitellogenesis without mating (Bona-Gallo and Licht, 1983; Garstka et al., 1985), and in a single study, 40–70% of unmated females proceeded through vitellogenesis (Whittier and Crews, 1986). These somewhat conflicting results may represent alternate physiological mechanisms or varying historical backgrounds of the snakes because the females were wild caught with unknown long-term histories.

Dependence of vitellogenesis on social interactions has not been directly studied in any other species of snake; however, correlations between mating and the female ovarian cycle have been made. Zwiefel (1980) noted that mating is normally a prerequisite to oviposition in *Lampropeltis getula*, whereas Alcobendas et al. (1992) observed that only female *Vipera aspis* that mated exhibited vitellogenesis. However, one of us (DFD) has observed two yearling female *Elaphe guttata* maintained in a room with males, but never having direct contact, oviposit infertile egg masses. Additionally, there is recent preliminary evidence for facultative parthenogenesis in bisexual snakes (Schuett et al., 1997), and such a phenomenon requires that vitellogenesis occur independent of male presence.

Even when correlations have been made between mating and female reproductive activity, the causation of this relationship is not understood. (That is, does mating stimulate female reproductive activity, or does female reproductive activity trigger mating? For an overview, see Naulleau et al., 1999.) Data from *V. aspis* demonstrates that reproduction is dependent on females reaching a minimum threshold in body condition (Naulleau and Bonnet, 1996), and only females that reach this threshold mate (Naulleau et al., 1999). Hence, progression through the reproductive cycle and mating is driven by the condition of the female.

These results may suggest that the regulation of reproduction is dramatically different between *V. aspis* and *P. curtus* (where the male induced changes in female reproductive condition). Alternatively, the combined results may demonstrate complimentary regulatory mechanisms present in a single system. A female's initial "decision" to reproduce in any given year may be driven by numerous physiological inputs including body condition, but progression through this process may be dependent on mating. Thus, mating may act as a permissive factor

of reproduction that assures fertilization and coordinates the reproductive process. In our study, given that all females were captive raised, in good body condition, and provided the necessary environmental stimuli for reproduction, all females were likely physiologically prepared to reproduce. However, the absence of a male prevented the progression of the reproductive process beyond the early, low-energy demanding stage of follicle hydration.

Reproduction is a complex and critical component of individual success. To properly address this vital component of life history, further studies must integrate physiological and ecological processes with phylogenetic patterns. Reproduction is not a single event but rather a series of behavioral and physiological events that all must occur to culminate in reproductive output. Mechanistic, physiological assessments including reproductive hormone level analysis and noninvasive, precise monitoring of the stages of reproductive activity are crucial for understanding the regulation of reproduction and in determining whether a general pattern of regulation exists in snakes.

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