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Sexual Conditioning in the Dyeing Poison Dart Frog (*Dendrobates tinctorius*)

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Amphibian populations worldwide are currently in decline. One approach to preventing extinction of some of the affected species is to create assurance colonies. These sustainable populations might some day be used to reestablish wild populations. One issue with creating assurance colonies is successful breeding; often difficulties arise when attempting to breed exotic animals in zoological institutions. Sexual conditioning, a form of Pavlovian conditioning, has been shown to improve breeding behavior. In this study the efficacy of sexual conditioning to improve breeding behavior in the dyeing dart frog (*Dendrobates tinctorius*) was tested. Pairs of frogs were exposed to one of three conditions. In two conditions pairs were trained with a stimulus (a flashing green light) that was either predictive of (experimental) or independent of (active control) exposure to a member of the opposite sex. The third condition was a no-treatment control. After training all three conditions were given five days to interact. Members of the experimental condition showed shorter latencies to a variety of breeding behaviors and produced more eggs than those in the control conditions. The sexual conditioning procedure was successful in increasing breeding behavior in this population of frogs.

Currently, world-wide, amphibian populations are in crisis. A large number of species are suffering catastrophic declines and widespread extinctions (Collins, 2010). As this decline continues researchers are searching for ways to preserve these species. While explanations for the cause of this decline may differ (e.g., Wake, 2007) there is little doubt that an extinction wave is occurring and something must be done, and soon. One suggested tactic is to collect animals from the wild before they go extinct and breed them ex-situ, which is currently the only recourse for some species that have already disappeared from the wild (Lips, Burrowes, Mendelson, & Parra-Olea, 2005). The goal of collecting these species would be to one day reestablish populations in the wild, once the causes for the decline can be ameliorated.

However, the value of ex-situ conservation is not unchallenged. Some question the wisdom of maintaining species for reintroduction when wild habitats are unlikely to be restored, zoological institutions tend to maintain breeding populations of species with low chances for repopulation in the wild, and examples of successful reintroductions are rare (e.g., Balmford, Mace, & Leader-Williams, 1996; Griffiths & Pavajeau, 2008; Reid & Zippel, 2008; Snyder et al., 1996). If the chances that the causes of the amphibian decline will be reversed are small, what value is there in maintaining these populations? However, even if reintroduction chances are slim, maintaining diverse amphibian populations under human care can have other benefits. Such populations may serve to educate the public, raise awareness of issues such as amphibian decline, or contribute to a wide range of research topics (e.g., Griffiths & Pavajeau, 2008; Leus, 2011; Moss & Esson, 2010; Reid & Zippel, 2008). Regardless of the reasons for maintaining ex-situ populations of these imperiled species, establishing assurance colonies would require the creation of breeding facilities (Gagliardo et al., 2008). However, the establishment of these facilities could prove challenging, as some species have proved difficult to maintain in zoological institutions and successful breeding can be elusive.

Breeding of exotic animals in zoological institutions is often problematic (e.g., Zhang, Swaisgood, & Zhang, 2004). During breeding attempts, animals may show inappropriate

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aggression, a lack of proper breeding behavior, or breeding attempts may prove unsuccessful due to infertility or unviable offspring (Augustus, Casavant, Troxel, Rieches, & Bercovitch, 2006; Bishop, Haigh, Marshall, & Tocher, 2009; Dalerum, Creel, & Hall, 2006; Munkwitz, Turner, Kershner, Farabaugh, & Heath, 2005; Swaisgood, Dickman, & White, 2006; Zhang et al., 2004). Consequently, practices that can address these issues would prove quite valuable in the breeding of exotics. Accordingly, some assisted reproductive technologies are already being perfected to be used on frogs due to difficulties in ex-situ breeding, including the cryopreservation of frog sperm and supplementing male and female frogs with hormones (Browne, Clulow, Mahony, & Clark, 1998).

However, the use of purely behavioral techniques is much rarer. Research on the use of behavioral techniques for the breeding of exotics appears to be an understudied area. This is unfortunate giving the myriad of different ways in which behavior plays an important role in successful breeding. Mate choice, recognition and preference as well as learning all play an important role in reproductive success (e.g., Kozak, Reisland, & Boughmann, 2008; South, Arnqvist, & Servedio, 2012; Verzijden, ten Cate, Servedio, Kozak, Boughman, & Evensson, 2012). Yet techniques that could exploit these knowledge areas are rare. One method that takes a behavioral approach and has not yet been attempted with amphibians is sexual conditioning.

Sexual Conditioning

Sexual conditioning, a form of Pavlovian conditioning, has been shown to improve breeding outcomes on a variety of measures. In this method, a stimulus that is initially ineffective at eliciting the target behavior (the conditional stimulus or CS) is repeatedly paired with exposure to a potential sexual partner (the unconditional stimulus or UCS). Exposure to the UCS then produces an unconditional response (UCR) in the subject. After repeated pairings of the UCS and CS the CS will come to elicit a conditional response (CR, which is similar to the UCR) independent of the UCS. In the culmination of the sexual conditioning procedure, after repeated pairings of the CS and the UCS, the animals are exposed to the CS and given a longer period of time to interact. The CS in this procedure, after training, may be thought of as a reliable predictor of breeding opportunities and the resulting CR may “prepare” the animal for mating, resulting in improved breeding behavior (Pfaus, Kippin, & Centeno, 2001).

Behavioral Changes in Sexual Conditioning

One of the most common outcomes seen in sexual conditioning is an increase in behaviors associated with breeding and a decrease in non-breeding related behaviors. Males in experimental conditions where access to a mate was reliably signaled showed more courtship behavior and less aggressive behavior towards females than males in the control condition (Domjan & Hollis, 1988; Hollis, Cadieux, & Colbert, 1989; Hollis, Martin, Cadieux, & Colbert, 1984). Sexual conditioning has also been shown to be effective in females. Female quail with reliably signaled encounters had positive changes in their breeding behavior as compared to control females (Gutiérrez & Domjan, 1997).

Sexual conditioning has been shown to improve breeding behavior in a variety of other ways. Other common outcomes are reductions in latency to copulation or ejaculation (e.g., De Jonge, Oldenburger, Louwerse, & Van De Poll, 1992; Domjan, Lyons, North, & Bruell, 1986). Sexual conditioning has also been used to improve the quality of breeding behavior. For example, rats that had previously failed to copulate, once exposed to the sexual conditioning contingencies, show an improvement in subsequent sexual performance (Cutmore & Zamble, 1988).

Sexual conditioning has also been shown to have direct reproductive benefits. For example, predictive signals have been shown to increase successful ejaculations and the amount and quality of sperm released by male rats, as well as the probability of fertilization of eggs in quail (Cutmore & Zamble, 1988; Domjan, Blesbois, & Williams, 1998). In fact, sexual conditioning can increase reproductive success in quail independent of which sex is being conditioned (Adkins-Regan & MacKillop, 2003). These effects can be demonstrated in fish as well, trained male blue gouramis spawned with females sooner, clasped females more often, and produced more young than control males (Hollis, 1990; Hollis, Pharr, Dumas, Britton, & Field, 1997).

Flexibility in Methodology

One extraordinary characteristic of sexual conditioning is its effectiveness despite large variations in methodology; this approach appears to be remarkably flexible. For example, male quail will respond to a sexual conditioning procedure with no appreciable differences in behavior even when the UCS (exposure to a female) differs in length from 30-240 seconds (Crawford & Domjan, 1993). Similar improvements in breeding behavior have been obtained independent of the size of the experimental chamber or variations in the CS-UCS interval (Domjan et al., 1986). The conditional stimuli successfully used for sexual conditioning have also varied widely. Stimuli have ranged from different sounds (Gutiérrez & Domjan, 1996), to visual access to a female (Hilliard & Domjan, 1995), to even a stuffed toy dog (Domjan, O'Vary, & Greene, 1988). Number of training trials can also vary, with successful conditioning achieved with as many as eighteen and as few as one CS-UCS pairings (Hollis et al., 1997; Hilliard, Nguyen, & Domjan, 1997).

Dyeing Dart Frogs

The subjects in this study were *Dendrobates tinctorius*, also known as the dyeing dart frog, members of the poison dart frog family. Several characteristics of this species make these frogs amenable to sexual conditioning. Dyeing dart frogs are relatively active (Lötters, Jungfer, Henkel, & Schmidt, 2007) and both sexes appear to play an active part in courtship. This allows for the potential of conditioning both sexes simultaneously and subsequently, improved breeding outcomes (Wells, 1977, 1978).

In this project the efficacy of sexual conditioning to improve breeding behavior in the dyeing dart frog (*Dendrobates tinctorius*) was tested. Pairs of frogs were either tested without any training (no-treatment control) or first trained with a stimulus that was either predictive of (experimental condition) or independent of (active control) exposure to a member of the opposite sex. It was expected that the subjects trained with the predictive stimulus would show shorter latencies to a variety of breeding behaviors and produce more eggs than subjects in either of the two control groups.

Method

Subjects

Subjects were 38 dyeing dart frogs (*Dendrobates tinctorius*), 19 males and 19 females, housed at the Atlanta Botanical Garden in Atlanta, Georgia. Subjects were paired based on sex and population morph (different populations can differ in coloration and/or pattern although they can still interbreed) and then randomly assigned to one of three conditions: a no-treatment control condition (nc), an active control condition (ac) and an experimental condition (exp). Both control conditions contained six pairs of frogs and the experimental condition contained seven pairs. Frogs were housed in conditions known to promote breeding behavior (personal communication, Robert Hill). They were kept at a photoperiod of 12:12, fed fruit flies three times per week and misted twice daily. Frog pairs were housed, one pair per tank, in ten gallon aquariums with metal mesh lids. The tanks were lined with a charcoal mix layer topped with sphagnum moss and included live plants and coconut breeding huts (Figure 1). Each hut contained a glass Petri dish, which has proven to be a suitable site for egg laying in this species (Pfeiffer, 2003). All frogs were given at least five days to habituate to the new tanks before training was started.

Figure 1. Tank configuration and an example of conditional approach behavior.

Materials

Each ten gallon tank contained a divider separating the two halves of the tank. The divider was opaque and could be raised through a slit in the lid (Figure 1). Green LED arrays were installed on the exterior of the tank, one on each side of the divider. Pairs of animals were housed with one animal on each side of the divider.

The CS used in this study was a flashing green LED. In an attempt to create a CS that this species would be sensitive to, the frequency of the flashing light was set to occur at a rate that approximated the speed of behaviors emitted by this species. Limb-shaking (behaviors described in Appendix), which is often observed during male-female interactions, occurs at a maximum rate of approximately two shakes per second (personal observation). Toe-trembling, which is correlated with feeding as well as male-female interactions, occurs at a maximum rate of approximately five vibrations per second (personal observation). The light was set to flash within this range, at a rate of approximately three flashes per second.

The UCS used was full access to the member of the opposite sex. Initial pilot study data suggested that visual access alone may produce aggressive rather than breeding behavior (data not shown). It is possible that tactile or olfactory cues are required for identification of sex between individuals in this species. As such, full access to the conspecific, rather than visual access, was employed as the UCS.

Procedure

In the experimental and active control conditions, each subject pair was exposed to five training trials per day for five days and both sexes were exposed simultaneously to the procedures. Each training trial consisted of a 90-s presentation of the CS and a 120-s presentation of the UCS. In the experimental condition the UCS immediately followed the CS and inter-trial intervals were randomly generated with a range of two to ten minutes. The lights would flash for 90 s and then the divider would be raised and the pair allowed to interact for 120 s. The times chosen were within ranges of past successful sexual conditioning procedures (Hollis, 1990; Pfaus et al., 2001).

Active control condition trials were conducted in accordance with Rescorla's (1967) suggested procedures for classical conditioning. For the control condition, timing of CS and UCS presentations were randomized separately, using the same two to ten minute time ranges. Using this procedure each trial is different in its timing, the CS may precede, succeed, or overlap with the UCS. CS and UCS presentations are independent. This allows for both the experimental and active control conditions to be exposed to both sets of stimuli for the same amount of time while separating them by the predictive value of the signal. This procedure equates many factors between the two conditions, preventing, among other things, misinterpretation of results due to pseudo-conditioning (May, 1949). Additionally, the CS for the active control condition must not be predictive of the UCS but it must also not be inhibitive, otherwise differences obtained may be inflated by comparing a CS+ with a CS- (e.g., Crawford & Domjan, 1996; Cutmore & Zamble, 1988).

After the five sets of training sessions had elapsed, testing trials were conducted. During testing trials the procedure for the active control and experimental conditions were identical. The CS was presented for 90 s after which the divider was raised allowing interaction between the pairs. The first hour of the testing trial was videotaped and the divider remained raised for five days. The tank was checked for eggs twice a day for five days. Testing for the no-treatment control condition pairs was the same except there was no presentation of the CS.

Scoring

Behaviors were observed using focal-group sampling observation methods (Altmann, 1974). Due to the small number in the group (two), their proximity to each other, and general restriction of location, both animals could be observed simultaneously; continuous focal observations of both animals were conducted without issue. Trials were scored on the amount and type of behavior exhibited by both sexes. For the testing trials, the latency to exhibition of various behaviors, the frequency of various behaviors, the latency to egg production, and the number of eggs produced were recorded for each pair.

The behaviors of interest were either identified during a pilot study or taken from descriptions of breeding behavior of related species. Behaviors were chosen based on the ability of the behaviors to be easily defined, differentiated, and potentially relevant to breeding behavior in this species. These behaviors are listed and described in the Appendix.

Location within the tank during training and testing trials was also recorded, as location within the testing area is a common measure of sexual conditioning. For example, the amount of time spent in the part of the cage containing the CS is a common measure for inferring response to the sexual conditioning procedure (Hilliard & Domjan, 1995). Across largely varying experimental conditions the most reliable indicator of successful sexual conditioning has been approach of the CS (Domjan et al., 1986). For this study each half of the tank was divided visually into eight equal parts, the horizontal plane was divided into four sections and the vertical plane into two (as this species spends a significant amount of time in the top half of their enclosure). Time spent in the eighth of the tank containing the CS during CS presentation was recorded.

Data Analysis

Locations within the tank, reproductive success, and frequency of and latency to breeding behaviors were examined. It was expected that, as training trials progressed, the subjects in the experimental condition would spend more time near the CS and show more breeding related behaviors during CS presentation than those in the active control condition. Additionally, it was expected that during the testing trials those in the experimental condition would show shorter latencies to breeding behavior, exhibit more appropriate breeding behaviors and lay more eggs, and lay them sooner, than those in either of the control conditions.

Non-parametric statistics were used for analysis as data collected during this experiment largely did not conform to the assumptions necessary for parametric statistics. As such, all values reported in this study are reported as medians. For comparisons between the experimental and active control conditions during training trials Mann-Whitney U, Wilcoxon signed ranks and trend tests were employed. Kruskal-Wallis tests were used to examine differences in testing trial data between the three conditions. Follow-up comparisons of testing data between pairs of conditions were analyzed using Mann-Whitney U tests. For pair-wise comparisons, comparisons between the experimental and either control condition were calculated as one-tailed while comparisons between control conditions were two-tailed. Significance for all test results was set at $p \leq .05$. Statistics were calculated either by hand or by using SPSS version 19.0.

Results

Training sessions

Behavioral Differences Between Conditions. During training trials, the behavior that showed the greatest difference between the experimental and active control conditions was calling behavior. The number of calls produced during the CS differed between the two conditions with those in the experimental condition producing, on average, significantly more calls during the CS period than those in the active control condition (11 vs. 0, Mann-Whitney U, $U = 6$, $p = .03$). Additionally, the number of calls produced during the UCS increased linearly over time in the experimental group, but not in the active control group (trend test, $z = 2.33$, $p < .01$) (Figure 2).

Location Differences Between Conditions. Conditional approach was observed in this experiment (Figure 1), the amount of time spent in the presence of the CS differed between conditions with the pairs in the experimental condition spending significantly more time near the CS during CS presentation than the active control pairs (1292 vs. 672.5 s, Mann-Whitney U, $U = 9$, $p = .05$).

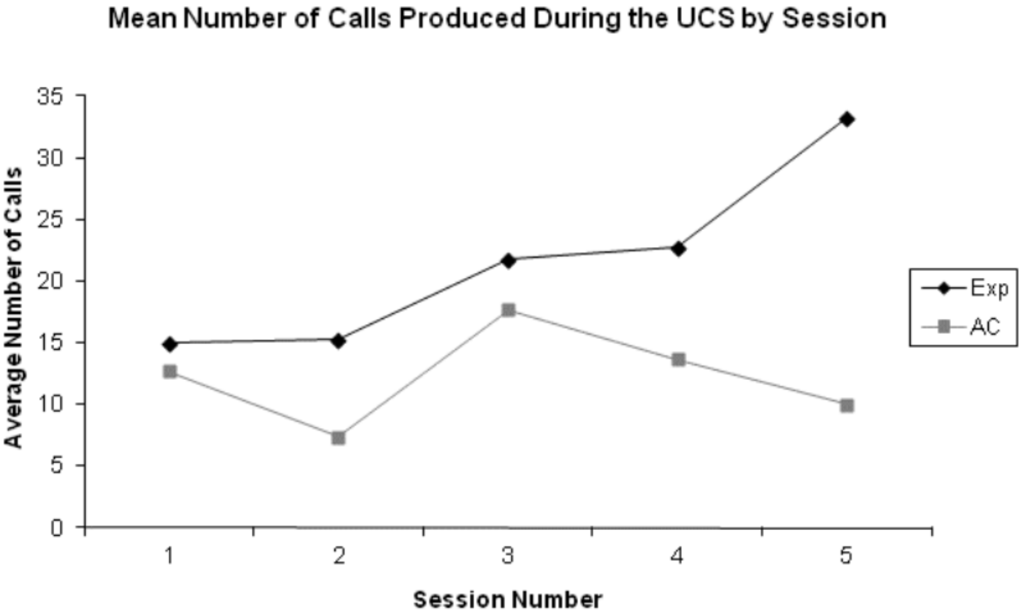

Figure 2. Mean number of calls produced during the UCS period of the training trials in the active control and experimental groups. Number of calls produced during the UCS increased linearly over time in the experimental group, but not in the active control group (trend test, $z = 2.33$, $p < .01$).

Testing Trials

Latency to Breeding Behaviors. One of the most consistent differences between the conditions during testing trials was differences in latencies to exhibit different breeding behaviors (Figure 3). For example, latency to first contact was significantly different between the conditions (EXP: 29.5, AC: 199, NC: 966.5 s, Kruskal-Wallis, $X^2 = 5.357$, $p = .001$) with preplanned comparisons demonstrating significant differences between all three conditions with the latencies being the shortest for the experimental condition and longest for the no-treatment control condition (Mann-Whitney U tests: EXP vs. AC, $U = 0$, $p = .002$; EXP vs. NC, $U = 0$, $p = .001$; AC vs. NC, $U = 3$, $p = .030$).

The latency to kneading behavior was also significantly different between conditions (Kruskal-Wallis, $X^2 = 9.36$, $p = .009$) with significant differences only between the experimental condition and the two control conditions with the experimental condition having shorter latencies (EXP: 164, AC: 957, NC: 1279 s, Mann-Whitney U tests: EXP vs. AC, $U = 1$, $p = .016$; EXP vs. NC, $U = 0$, $p = .004$; AC vs. NC, $U = 7$, $p = .310$) (Figure 3).

Differences were also apparent in the latency for the two sexes to approach each other (Figure 3). The latency to male pursuit of the female differs (EXP: 24.5, AC: 190, NC: 745.5 s, Kruskal-Wallis, $X^2 = 14.235$, $p = .001$) with significant differences between all three conditions with the experimental condition having the lowest latencies and the no-treatment control condition the highest (Mann-Whitney U tests: EXP vs. AC, $U = 0$, $p = .002$; EXP vs. NC, $U = 0$, $p = .001$; AC vs. NC, $U = 0$, $p = .004$). Latency to pursuit by female also differs (EXP: 43.5, AC: 388, NC: 1093.5 s, Kruskal-Wallis, $X^2 = 12.74$, $p = .002$) with significant differences only between the experimental condition and the two control conditions with lower latencies in the experimental condition (Mann-Whitney U tests: EXP vs. AC, $U = 0$, $p = .002$; EXP vs. NC, $U = 0$, $p = .001$; AC vs. NC, $U = 4$, $p = .052$).

Additionally, only the experimental condition had differences in pursuit that were grouped significantly in a non-random manner (Figure 4) (Chi-square: EXP: $X^2 = 5.444$, $p = .020$). All pairs in the experimental condition had latency to male and female pursuits that were within one minute of each other. In all experimental pairs if one member of the pair pursued the other, that pursuit was reciprocated within 60 s.

Egg Production. In the experimental condition four pairs produced eggs (median: 4 eggs). In both control conditions, only one pair produced eggs (2 eggs each). The number of eggs produced in the three conditions were significantly different (Kruskal-Wallis, $X^2 = 5.987$, $p = .05$) with the experimental condition producing significantly more eggs than the two control conditions (Mann-Whitney U tests: EXP vs. AC, $U = 8.5$, $p = .036$; EXP vs. NC, U = 8.5, $p = .036$; AC vs. NC, $U = 18$, $p = 1.0$). There was little variability in latency to egg production, 67% of eggs were found on the morning of the third day. The other two sets were found on the evening of the second day and the evening of the fourth day and were both laid by experimental pairs.

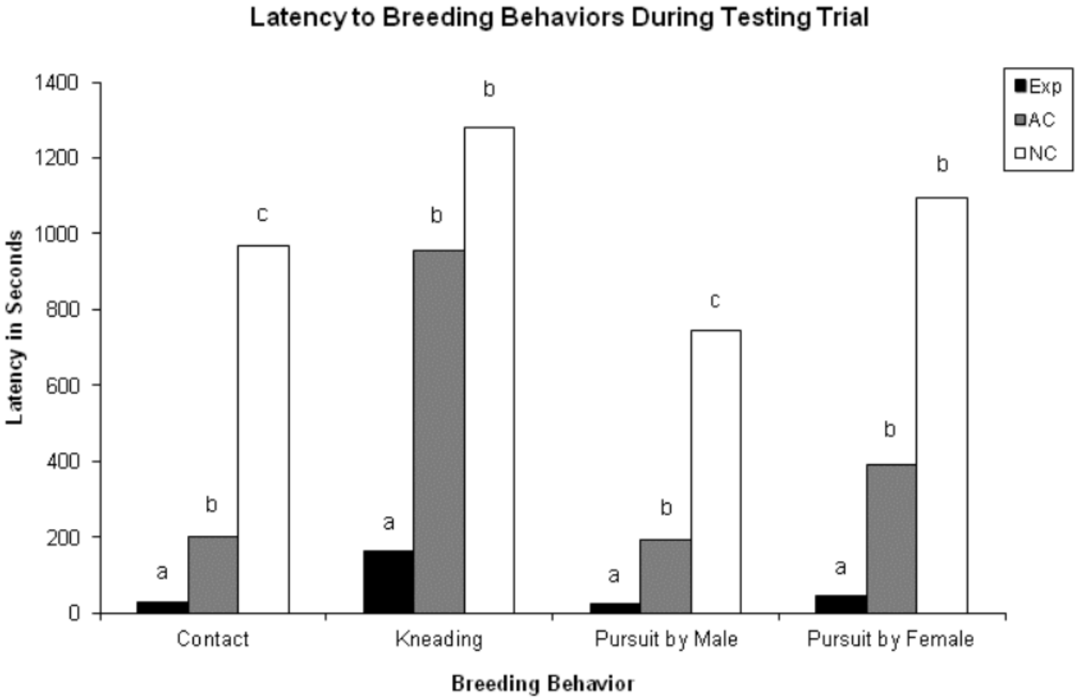

Figure 3. Differences in latencies to behavior in the three conditions. Within each behavior having different letters denotes a significant difference between the groups, $p \leq .05$. Each bar represents median values for each group.

Figure 4. Symmetry of pursuit behavior, *denotes distribution is not uniform, $p < .05$.

Discussion

In this experiment, the sexual conditioning procedure was shown to be successful at improving breeding behavior in an amphibian species. The number of eggs produced in the three conditions was significantly different with the experimental condition producing more eggs than the two control conditions. However, sexual conditioning in this species did not result in decreased latency to reproduction, as has been demonstrated in some other species (Hollis et al., 1997).

In this study, both control conditions produced similar numbers of eggs. However, on several latency measures the active control condition showed significantly reduced time to breeding behaviors than the no-treatment control condition. Consequently, the important behaviors of interest in this procedure should then be the measures that do not differ significantly between the control conditions but do differ in the experimental condition. Two measures most clearly fit these criteria. Latency to kneading behavior and latency to pursuit by the female were significantly different only between the experimental condition and the two control conditions. Other measures had data suggestive of this pattern. For example, the time spent within one frog length and latency to call show a similar pattern, although the differences are not significant (data not shown).

Another measure that differed only in the experimental condition is symmetry of pursuit latencies. Only the experimental condition had differences in pursuit that were grouped significantly in a non-random manner. All differences were 60 s or less whereas in the two control conditions differences in pursuit were as long as ten minutes.

Using Sexual Conditioning to Study Breeding Behavior

Beyond improved reproductive success, another benefit of the sexual conditioning procedure is that breeding behaviors may be more easily studied. This procedure condenses the period of time during which breeding behavior can be expressed, and increases the frequency of the behaviors as well, allowing for easier observation.

One example of this experimentally increased behavior is calling. Males in the experimental condition produced significantly more calls during the CS period than those in the active control condition and the number of calls produced during the UCS increased over time in the experimental condition, but not in the active control condition. Having calling behavior produced in a predictable manner allows for easier study. As an offshoot of this project high-quality recordings of breeding calls for this species were obtained for quantitative analysis (Gaalema & DuPée, in preparation).

Breeding of Exotics in Zoological Facilities

The encouraging results obtained in this study have important implications for the breeding of exotics in human care. As mentioned previously, the breeding of exotics is often a difficult process with a myriad of potential issues. Zoological facilities are not the optimal context for the breeding of exotic animals and success can be very low (e.g., McDougall, Réale, Sol, & Reader, 2006). One of the most common hurdles in breeding exotic animals is a lack of proper breeding behavior (Munkwitz et al., 2005; Zhang et al., 2004). Inappropriate breeding behavior has been implicated as a major problem in successful breeding in a variety of species from birds to pandas. As such, overcoming these behavioral issues is a major objective for zoological facilities. Accordingly, a procedure with the potential to increase reproductive success could have wide-spread application.

Results from this study provide additional support to earlier sexual conditioning studies that have shown that Pavlovian conditioning could be effective in battling many of the aforementioned issues. Not only has sexual conditioning been shown to promote positive breeding behaviors and decrease aggressive behaviors (e.g., Cutmore & Zamble, 1988; Hollis et al., 1989) but it has also been shown to increase the number of viable offspring produced in a successful encounter (Hollis, 1990). These earlier studies, combined with the current data, suggest that indeed sexual conditioning might prove a successful tool for improving breeding behavior for exotics in human care.

Anecdotal evidence suggests that this procedure may have additional lasting benefits. Pairs in the experimental condition were reported by animal staff (who were blind to which animals had been exposed to which contingencies) to be spending more time in proximity to one another and showing increased incidences of breeding behavior as compared to prior to the experiment. Additionally, several frogs that had never produced eggs prior to the study have now begun to lay eggs on a regular basis. It appears as if the procedure may have additional benefits of facilitating introductions, or possibly fostering long-term breeding relationships between individuals.

Amphibian Decline

Improving breeding success is especially important for amphibian populations. As previously mentioned, amphibian numbers are in decline and action must be taken quickly to prevent an extreme loss of diversity (Collins, 2010). Creation of assurance populations requires breeding these amphibians on a large scale, and in a relatively short period of time. The advantages gleaned from sexual conditioning could be particularly useful for the creation of amphibian assurance colonies, where time is of the utmost importance. The results from this study provide support for the utility of sexual conditioning for promoting reproduction in amphibians in human care. The current technique could be useful in species where hormone manipulations have only produced infertile eggs (e.g., Bishop et al., 2009) or this technique could be combined with others, potentially further improving breeding outcomes (e.g., Browne et al., 1998).

Overall, this procedure has proved successful at increasing reproductive success in *D. tinctorius*. Sexual conditioning, which is known to be very flexible in its application, appears to be effective in amphibian species as well. This extension to amphibians provides evidence that this procedure could also potentially generalize to a variety of other species. In addition to increased reproductive success, sexual conditioning also increased the expression of breeding behaviors. This increased expression, along with the condensed time in which it occurs, could allow for easier observation of breeding behavior in any species of interest. Sexual conditioning is an area rife with opportunity for continued study in the areas of the breeding of exotics or the study of sexual behavior.

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Appendix
Behavior Descriptions

Contact: Physical contact occurs between the two frogs.

Pursuit: Frog moves within one body length of other frog (male and female) or frog commences calling upon seeing other frog (male only).

Call: Only seen in males. Body of frog inflates and deflates, accompanied by a rasping sound. Throat sac may or may not inflate.

Male mount: Male places his ventral surface on female's dorsal surface with both heads oriented in the same direction.

Kneading: Female raises and lowers her front feet, either individually or in succession, rhythmically and repeatedly. The feet may be resting on the male or on substrate directly adjacent to him. Movement is slower than in limb-shaking.

Toe-trembling: Fourth toe of back foot vibrates up and down rapidly. (Hödl & Amézquita, 2001)

Limb-shake: Front or back foot is raised and lowered quickly (Hödl & Amézquita, 2001).

Body-lowering (Crouching): Either whole body or anterior part of the body is pressed against the substrate (Hödl & Amézquita, 2001).

Upright posture: Arms are extended and the anterior part of the body raised (Hödl & Amézquita, 2001).

Stroke: Frog touches other frog with front foot.