

**The role of female breeding coloration in the behavior and mate selection of male leopard lizards (*Gambelia wislizenii*)**

*MS Thesis Proposal*

**Kamelia Fallahpour**

*Laboratory of Integrative and Comparative Herpetology*

Department of Biology

California State University, Northridge

*Abstract*

I will determine how female breeding coloration (FBC) affects mate choice by male long-nosed leopard lizards, *Gambelia wislizenii*. Among animals, visual signals are an important component of social behavior and have important fitness consequences. For example, brightly colored individuals may have higher mating success, but this comes at the cost of higher predation. There are also metabolic costs associated with synthesizing the pigments that account for the color change. Although rare, some female vertebrates exhibit secondary coloration during the breeding season. In lizards, this coloration may be present in gravid females and is associated with aggression towards males; however, the relationship between FBC and mate selection is poorly understood. Reports on the relationship between FBC and mate selection in leopard lizards are anecdotal, conflicting, and have been uncritically perpetuated in popular field guides. My study seeks to replace these anecdotes with experimentally derived data. I will test two hypotheses for the role of FBC in leopard lizards: (1) FBC is not a signal to male lizards, thus males should attempt to copulate with females with and without FBC equally; (2) FBC is a signal to other females, thus females may avoid plain-colored conspecifics (i.e., males) in the mating season because they are frequently harassed by males or forced to copulate with them.

My study integrates the fields of physiology and behavioral ecology and has several components. First, I will implant progesterone in females to induce breeding coloration and testosterone in males to induce breeding behaviors. I use videotape to record the frequency and nature of behaviors exhibited by both sexes as females develop breeding coloration. Next, I will isolate the effect of behavior from color in a color-manipulation experiment by giving males a choice of a plain female (control) or a painted female (matching FBC). Third, I will record behavioral responses of males given a choice between females with and without breeding coloration in the lab and field. Last, I will examine female responses to other females with and without FBC to address the hypothesis that FBC is a female-female signal.

My preliminary data (from hormone implantations and color-manipulations) suggest that FBC in *G. wislizenii* is not a signal to males, as previously reported for this species and for most other iguanian lizards. Preliminary data also suggests that FBC may be a signal to female conspecifics. Documenting the behaviors associated with FBC in these lizards will lead to a deeper understanding of the role of this coloration in intersexual communication specifically, and in visual signals used by lizards generally.

### *Background*

Communication between organisms is achieved by diverse types of signals including those that involve chemical, auditory, visual, and tactile cues. Among animals, visual signals can be an important component of social behavior and often has important fitness consequences. For example, brightly colored individuals may have higher mating success, but this can come at the cost of higher predation (Andersson 1994; Fox et al. 2003). Coloration is a type of visual signal that is used by many species of lizards. Although bright breeding coloration is common in male lizards, it is rare in females (Cooper, 1988). Bright female coloration, although uncommon, is taxonomically widespread in iguanian lizards and influences mate choice in both sexes (Cooper, 1988). Breeding coloration may have important fitness consequences in female lizards. In particular, gravid individuals may have a higher risk of predation because the bright coloration makes these otherwise cryptic animals more conspicuous (Watkins, 1997). There are also metabolic costs associated with synthesizing the pigments that account for the color change (Cooper and Greenberg, 1992). Yet, female breeding coloration has independently evolved in more than 30 species of iguanian lizards (Cooper and Greenberg, 1992). Thus, the signal communicated via female breeding coloration must have important fitness benefits that outweigh its costs.

Secondary sexual coloration is typically exhibited during the breeding season. During this time, mature females develop vibrant colors (usually yellow, orange, or red) on certain areas of their body. This coloration is frequently present in gravid females and is often associated with agonistic behavior towards males. Of the 30 species of lizards with secondary sexual coloration, 19 are known to perform aggressive rejection displays during the season when they exhibit breeding coloration (Cooper and Greenberg, 1992).

### *Relationship between the Reproductive Cycle & Breeding Coloration*

Breeding coloration, and its associated behaviors, has been studied in six species of iguanian lizards (Cooper and Greenberg, 1992; Hager, 2001). Breeding coloration and aggressive behaviors are closely correlated with hormone levels, particularly high levels of progesterone (Medica et al., 1973). Most iguanian lizards that exhibit secondary sexual coloration develop this coloration during ovulation, which is when eggs are being formed in the ovaries (Ferguson, 1976). Females typically copulate before or soon after they exhibit peak breeding coloration, but some species are only receptive prior to exhibiting breeding coloration and become aggressive after they have brightened (Cooper and McGuire, 1993). Still others (e.g., *Holbrookia maculata*) are receptive while exhibiting a low-intensity coloration, and become aggressive later in the season as the intensity peaks, signaling that they are gravid and thus no longer receptive (Hager, 2001). Females usually retain breeding coloration until oviposition, after which the color gradually fades over a period of days or weeks (Cooper and Crews, 1988).

Female leopard lizards (*G. wislizenii*) develop breeding coloration during vitellogenesis, the stage prior to ovulation when the yolk is being synthesized (Medica et al., 1973). In these lizards, copulation and fertilization take place during ovulation (Cooper and Clarke, 1983). After oviposition, females gradually return to their normal cryptic coloration. Breeding coloration in female *G. wislizenii* develops as vibrant red-orange spots and streaks on the sides of the head, body, and ventral surface of the tail (Medica et al., 1973). Females present this coloration in aggressive encounters with males during which females sidle hop and back arch flashing their orange color (Montanucci, 1965).

### *Hypotheses Proposed for the Role of Breeding Coloration*

Aggressive behavior is frequently associated with bright coloration in female iguanian lizards, but the function of the change in color is poorly understood. In most lizard species, breeding coloration is exhibited by the female just before she is gravid, and thus may play a role in advertising receptivity (e.g., Cooper and McGuire, 1993). But females of other species only exhibit breeding coloration after they have become gravid, perhaps signaling that they are no longer receptive. Aggressive behavior by females exhibiting breeding coloration may be an additional cue signaling that they are gravid and no longer receptive. Yet, other species have less vibrant coloration when they are receptive and develop a more intense coloration only after they are gravid (e.g., Hager, 2001). Hypotheses that have been proposed to explain female color change include sex recognition, female signaling, aggressive avoidance, courtship rejection, courtship stimulation, and conditional signaling (reviewed by Cooper and Greenberg, 1992). However, none of these single hypotheses entirely explains the role of breeding coloration for all species. Below I review these hypotheses and discuss whether they are applicable to the breeding coloration exhibited by female *G. wislizenii*.

The **sex-recognition hypothesis** suggests that breeding coloration in female iguanian lizards evolved as a signal for males to recognize females during the mating season. Male *Holbrookia propinqua* are less aggressive towards males painted to resemble females exhibiting breeding coloration than they are to males lacking the coloration (Cooper, 1984, 1986). This suggests that female breeding coloration functions in sex recognition. Yet, other female iguanian lizards that lack breeding coloration are recognized by both males and females on the basis of sexually dimorphic traits or behavioral and pheromonal cues. Male *H. propinqua*, for example, are larger and more aggressive than females, and have a color pattern that is different from

female color patterns in both receptive and unreceptive females. Yet, female *H. propinqua* display breeding coloration, indicating the color has a purpose other than sex recognition. Breeding coloration does not seem to be a signal for sex recognition in *G. wislizenii* because females display behaviors (e.g. side-hopping, back arching, etc.) that are not exhibited by males (personal observation). Thus, males and females can be recognized easily by behavior. Also, female *G. wislizenii* only exhibit breeding coloration during certain periods of the breeding season, and the color fades and later re-intensifies (Medica et al. 1973). If breeding coloration in *G. wislizenii* is a sex-recognition signal, females should exhibit the same intensity of breeding coloration during the breeding season, or throughout the year. Thus, breeding coloration must serve a purpose other than sex recognition.

The **female-signal hypothesis** posits that female breeding coloration is used as a social signal to other females of the same species. This hypothesis may be supported for *G. wislizenii* because preliminary results show that females are more tolerant of other females with breeding coloration than plain-colored conspecifics (i.e., males) during the mating season because females are frequently harassed by males and even forced to copulate with them (Moore, 1983). In contrast, female *H. propinqua* exhibiting and lacking breeding coloration are equally aggressive to other females, whether they exhibit breeding coloration or not (Cooper and Greenberg, 1992). Females exhibiting breeding coloration also have specific courtship rejection behaviors such as side hopping and back arching that are displayed only to males. Thus, female breeding coloration may serve more than one role: (1) male courtship rejection and (2) female-female signaling. For *G. wislizenii*, a limitation of this hypothesis is that breeding coloration fades between clutches (Medica et al., 1973), so females cannot provide constant signals. Breeding coloration might also be a signal to females if they compete for or defend nesting sites. Yet, in

habitats that have been studied, individual *G. wislizenii* do not live in close proximity with conspecifics and appear to lack territories (Tollestrup, 1983). This suggests that competition for nest sites may be unlikely particularly if female-female encounter rates are low. Thus, breeding coloration appears to serve a purpose other than female-female recognition in *G. wislizenii*.

The **aggression-avoidance hypothesis** states that breeding coloration is a signal to males indicating that females are gravid. Under this model, breeding coloration would prevent males from exhibiting aggressive courting behaviors toward gravid and aggressive females. Because females lacking breeding coloration are not aggressive towards males, they do not need the coloration. Yet, aggressive behaviors of females exhibiting breeding coloration are distinct from aggressive behaviors of males (Cooper and Greenberg, 1992). Thus, breeding coloration is not necessary to distinguish the gravid female from a receptive female, because females have distinct aggressive behaviors signaling to males that they are gravid. Also, males do not always reciprocate aggressive behavior toward aggressive female, regardless of the presence of breeding coloration (Cooper and Greenberg, 1992). Thus, aggression avoidance may not be a widely applicable explanation for the evolution of breeding coloration. For *G. wislizenii*, breeding coloration develops before ovulation, and thus females already exhibit breeding coloration prior to mating. Hence, breeding coloration cannot be a signal that females are gravid. Also, male *G. wislizenii* are not consistently aggressive toward females, regardless of female coloration (personal observation). Thus, breeding coloration must serve a different purpose than aggressive avoidance in *G. wislizenii*.

According to the **courtship-rejection hypothesis**, females exhibit breeding coloration to deter males from courting them when they are gravid. This hypothesis explains why some females are aggressive and reject courtship from males when they exhibit breeding coloration,

and why this coloration is retained the entire time the female is gravid. Thus, breeding coloration is a signal that the female is not receptive, and males should court only females that lack this coloration. The limitation of this hypothesis is that, in some species, females that have developed (or are developing) breeding coloration do not reject courtship. In fact, in some species, females are receptive and allow mating after they have developed breeding coloration (Cooper and Greenberg, 1992). Additionally, males are not more aggressive toward females that lack breeding coloration (Cooper and Greenberg, 1992). In *G. wislizenii*, females develop breeding coloration before ovulation, so breeding coloration is unlikely to be a signal that the females are not receptive. Also, equal numbers of copulations have been reported for female *G. wislizenii* exhibiting breeding coloration as there have been for females lacking breeding coloration (see section: Role of Breeding Coloration in *Gambelia wislizenii*). Thus, males mate with females exhibiting breeding coloration just as readily as plain individuals. Therefore, this hypothesis does not account for the role of breeding coloration in female *G. wislizenii*.

The **courtship-stimulation hypothesis** posits that females exhibiting breeding coloration are advertising that they are receptive for fertilization. Females that have recently begun to develop breeding coloration are sexually receptive and most begin to exhibit their brightest breeding coloration in the final stages of vitellogenesis. *Holbrookia propinqua* and *G. wislizenii* both begin to exhibit breeding coloration prior to ovulation. *Holbrookia propinqua* are not aggressive when they begin to exhibit breeding coloration, but become aggressive within a day after copulation (Cooper and Crews, 1988). However, in some species, females lacking breeding coloration are also sometimes receptive. Females of some species, including *G. wislizenii*, retain breeding coloration while gravid and no longer receptive, yet do not lose their coloration until after oviposition. Also, equal numbers of copulations have been reported for female *G. wislizenii*



exhibiting breeding coloration as there have been for females lacking breeding coloration (see section: Role of Breeding Coloration in *Gambelia wislizenii*). Thus, males mate with females exhibiting breeding coloration just as readily as plain individuals. Therefore, this hypothesis does not account for the role of breeding coloration in female *G. wislizenii*.

The **conditional-signal hypothesis** suggests that female receptivity varies with changes in the relative intensity of the breeding coloration. Low-intensity coloration in the early breeding season signals receptivity in the female, whereas high-intensity coloration later in the breeding (when females are gravid) season signals courtship rejection (Cooper and Greenberg, 1992; Hager, 2001). This hypothesis may explain the role of female coloration in territorial species, in which males monitor the intensity of female coloration. But *Gambelia* are considered to be nomadic not territorial (Tollestrup, 1983). Consequently, it is unlikely that roaming males could continuously monitor the intensity of breeding coloration of specific females. Nevertheless, males and females have been found in pairs during the reproductive season (May–July; Montanucci, 1965), suggesting that males may court females for extended periods. Perhaps these pairings are cued by changes in the intensity of female breeding coloration. However, the conditional-signal hypothesis does not account for male *Gambelia* courting females that lack breeding coloration (Medica et al., 1973), or captive males copulating with both plain and brightly colored females (Montanucci, 1965).

In summary, none of these individual hypotheses explain the evolution and the role of breeding coloration in female *G. wislizenii*. It is possible that some combination of these hypotheses may explain this role, or that the factors that are historically responsible for the evolution of breeding coloration in *G. wislizenii* are no longer present in extant populations.

*Role of Breeding Coloration in Gambelia wislizenii*

For *G. wislizenii*, the relationship between female breeding coloration and reproductive receptivity is unclear. Some researchers have observed females lacking breeding coloration to be receptive and females exhibiting breeding coloration to be aggressive (Cooper, 1984; Cooper and Greenberg, 1992), but others state that females lacking breeding coloration are as receptive as females exhibiting breeding coloration (Montanucci, 1965; Medica et al., 1973). This controversy stems from the anecdotal basis of these reports.

Anecdotal evidence suggests that receptive female leopard lizards are relatively passive towards males compared to other iguanian lizards, but gravid females are aggressive towards males and may even bite them (Montanucci, 1965). Nevertheless, it is unclear how the color change of females affects the behavior of and mate selection by males. Two females exhibiting breeding coloration have been observed copulating in nature, but two females lacking breeding coloration have also been observed mating (Medica et al., 1973). Moreover, captive males in the absence of choice will copulate with brightly colored females in the same frequency as with females lacking breeding coloration (Montanucci, 1965).

These anecdotes appear to have been misinterpreted in the literature. Cooper and Greenberg (1992; their table 6.9) state *G. wislizenii* (and *G. sila*) exhibit aggressive courtship rejection displays such as back arching, sidle hopping, and bright female coloration. They also list female *Gambelia* as reaching the peak of their brightness when they are gravid. Sources for this information are cited as Montanucci (1965, 1967) and Medica et al. (1973). Montanucci (1965; p. 279) states, "Postnuptial coloration is assumed a week after copulation, the color becoming vivid within 2 weeks. The functional significance of this coloration is not evident. Applegarth (pers. comm.) suggests that it serves to deter copulation." This statement suggests

that breeding coloration plays a role in courtship rejection, but the next sentence states, “I have observed captive males copulating as readily, if not more so, with marked as with unmarked females, indicating that the color is likely to serve as a releasing stimulus.” If males prefer to court females exhibiting breeding coloration than females without breeding coloration, this coloration may be a signal for courtship stimulation. Montanucci (1967; p. 120–121) discusses only the non-receptive behaviors of females, and for receptive females he states “The role of the female in mating is generally passive. In one instance, however, a female *wislizenii* moved nearer to and licked the male prior to copulation.” Medica et al. (1973; p. 660) are also cited as evidence for a relationship between female breeding coloration and receptiveness in *G.*

*wislizenii*:

... we have observed a brightly colored female *C. wislizenii* copulating in southern Nevada, as has been observed elsewhere (Montanucci, 1967; Mayhew, 1968)... Two uncolored female *Crotaphytus wislizenii* from southern California were observed copulating. The brightly colored female observed copulating in southern California by Mayhew (1967) was captured on 3 May 1958, and deposited eggs on 27 May 1958 (Mayhew, pers. comm.).

From these anecdotes, Medica et al. (1973; p. 660) conclude:

...this lizard probably contained enlarged yolked follicles at the time of copulation. Under these circumstances, the coloration probably functions not only in sex recognition, but also as an attractant to male lizards at a time propitious for fertilization of the maturing ova. Montanucci's observations (1965) of males courting colored females support this hypothesis.

What is the relationship between female breeding coloration and behavior in *G. wislizenii*? Some authors have asserted that female *G. wislizenii* are aggressive when brightly colored, but an equal number of observations (although anecdotal) suggest that they can be receptive when they are bright. There has also been an anecdotal observation of a gravid female

lacking breeding coloration (L. Iacovelli, pers. comm.). It is possible that, like *H. propinqua*, *G. wislizenii* copulates after achieving its brightest coloration, thereafter becoming aggressive, but this hypothesis does not account for observations of copulating females lacking breeding coloration (Cooper and Greenberg, 1992). It is also possible that female *G. wislizenii* are receptive when they begin to exhibit breeding coloration, but become aggressive as the color intensifies to signal that she is gravid, as in *H. maculata* (Hager, 2001). Additionally, breeding coloration fades in leopard lizards after a clutch is deposited, yet re-intensifies for the second clutch (Medica et al., 1973) suggesting that the color is correlated with being gravid.

Unexpectedly, breeding coloration in *G. wislizenii* could be a signal to mature females, rather than to males. Preliminary studies suggest that females with breeding coloration are more tolerant of other females with breeding coloration than females lacking breeding coloration (Moore, 1983). Hence it is proposed that potentially high cost of the high frequency of forced courtship and mating has selected for females that can recognize and avoid males. Thus, females should avoid or be aggressive towards all males and females lacking breeding coloration, which resemble males. In Moore's (1983) study, females lacking or exhibiting breeding coloration were introduced to other females. The conclusions drawn from this study are limited by the small sample sizes ( $N = 6$ ) and the fact that females were not introduced to males to test whether female breeding coloration provides a signal to males.

The discrepancies among literature reports on the role of female breeding coloration in *G. wislizenii* are likely attributable to the anecdotal nature of these reports, which typically involved only one or two animals and chance observations. My study will be the first to experimentally examine the effects of female breeding coloration on male behavior and mate selection in *G.*

*wislizenii*. Thus the results of my investigation will resolve this 30-year controversy by replacing anecdotal reports with empirical data.

### *Objectives*

I will determine the role of female breeding coloration and associated reproductive behaviors in female and male long-nosed leopard lizards, *G. wislizenii*. As noted above, reports of the role of breeding coloration in this species are anecdotal (one or two individuals), conflicting, and have been uncritically perpetuated in popular field guides (e.g., Behler and King, 1979). My study will be the first to investigate the behavior of female *G. wislizenii* at times when they are exhibiting breeding coloration and the first to examine the effects that this coloration has on male behavior and mate selection. My investigation will replace anecdotal reports with empirical data.

### *My Objectives & Predictions*

To develop an understanding of the function of female breeding coloration in *G. wislizenii*, it is critical to determine whether this coloration is a signal to males. Behavioral observations of female *G. wislizenii* exhibiting and lacking breeding coloration are needed to determine the relationship between breeding coloration and female receptiveness, and how males respond to the color and behavior of females. Color intensity should also be examined to determine whether females are receptive when exhibiting low-intensity coloration and become aggressive with high-intensity coloration.

I have several predictions about the behavior of males towards female leopard lizards with and without breeding coloration: (1) males will court females lacking breeding coloration (and females painted as such) and will be non-receptive or aggressive towards females exhibiting

breeding coloration (and females painted as such). If so, breeding coloration in leopard lizards is a courtship-rejection or an aggression-avoidance signal, informing males that the female is not receptive or gravid; (2) males will court female leopard lizards exhibiting breeding coloration (and females painted as such), but will be non-receptive or aggressive towards females lacking breeding coloration (and females painted as such). If so, breeding coloration plays a role in sex recognition or courtship stimulation, signaling to males that the individual is a female and/or that she is receptive; (3) males will be receptive towards females regardless of female coloration. If this hypothesis is supported, then breeding coloration in female leopard lizards is not a signal to males during the reproductive season. These experiments will clarify male preference for females lacking and exhibiting breeding coloration, changes in behavior as females develop breeding coloration, and the use of breeding coloration as a signal of female receptivity to males.

To determine whether female breeding coloration in *Gambelia wislizenii* is a signal to males, I will (1) monitor male behavior toward females to determine at what point males change their behavior from receptive to aggressive (or avoidance) as females develop breeding coloration, (2) monitor the difference between male behavior towards females exhibiting and lacking breeding coloration in the lab, (3) determine male preference for females with breeding coloration using color manipulation, and (4) quantify male behavior toward females exhibiting and lacking breeding coloration in the field. I will also determine whether breeding coloration is a signal to females by (1) monitoring female behavior toward females exhibiting and lacking breeding coloration in the lab and field, and (2) using color manipulation to determine whether females (exhibiting and lacking breeding coloration) are attracted, deterred, or even respond to females lacking and exhibiting breeding coloration.

## Materials and Methods

My study has four primary components: (1) hormone implantation to induce breeding coloration and male receptivity, (2) color manipulations in which both males and females are making mate choices, (3) male preference towards females exhibiting and lacking breeding coloration during the breeding season in the laboratory and in the field, and (4) color manipulation and field experiments using only females to determine if breeding coloration is a signal to female conspecifics.

### *Animals and their Care*

Fifteen adult *G. wislizenii* (14 females and 5 males) were collected near Fallon, NV in August 2002 and purchased from a commercial collector. An additional 23 adults (15 females and eight males) were collected in Hawthorne, Nevada on 2–4 June 2003. All lizards were brought to the Department of Biology Vivarium at California State University, Northridge, weighed, measured (body length), toe-clipped for identification, and briefly soaked in vegetable oil to remove external parasites (Espinoza et al. 1998). Lizards are housed individually in glass aquaria (25 x 50 x 30 cm) and visually isolated from each other by covering the sides of each aquarium with paper. The enclosures are lined with sand (3–4 cm) and heated from 0.5 m above by a 125-W heat lamp. Ultraviolet fluorescent lights provide a 14L:10D photocycle (during activity season), and ceramic pots and PVC pipe provide refugia. Water is available ad libitum and the animals are fed ad libitum portions of crickets (*Acheta domesticus*) or mealworms (larval *Tenebrio molitor*) twice weekly. Food items were dusted with calcium and vitamin powder weekly. During experiments, females were given 1–2 drops of carotene red (0.1% solution; Curtis's Canaries, Plano, TX) orally each week to insure that the pigments putatively responsible for

breeding coloration were available. Animals were weighed monthly to monitor health. All lizards either maintained body mass or grew slightly over the course of the experiments.

### *Hormone Implantation*

This portion of the experiment will determine whether male behavior changes towards females as females develop breeding coloration. I will monitor female coloration and male behavior towards focal females on a daily or every-other-day basis.

A 4 x 2-m wooden arena was constructed for use in the lab. The arena was lined with 2 cm of sand and was heated from above (0.5 m) by four 250-W and four 125-W heat lamps. A black plastic curtain was used to hide the observer from the lizard's view (Sugerman 1980).

Time-release hormones (Innovative Research of America, Sarasota, FL) were implanted in five males and five females in 2003, and in six males and six females in 2004. Dosages were as follows: 1.5 mg, 21-day release progesterone for females and 5.0 mg, 21-day release testosterone for males. Placebos were also implanted in one female in 2003, and 6 females and 5 males in 2004.

Tablets (0.0177g; 3.33mm) were implanted subcutaneously along the skin folds of the neck or dorsal surface of the thigh 2–3 min following injection of a local anesthetic (0.5 ml of 0.5% lidocaine HCl). Following implantation, the wound was swabbed with Fougera<sup>®</sup> triple antibiotic ointment (E. Fougera & Co. Melville, NY). The wounds healed within 3–7 d.

Lizard pairs were randomly assigned a priori and behaviors were recorded in the arena via videotape for 3 min (Hager, 2001) starting the day after implantation. If copulation occurred, the trial was extended (by videotaping or observation) for another 20–30 min, or until mating ceased. Recording began when either sex acknowledged the presence of the other by



approaching or displaying (e.g., pushups). If no interaction was observed for 10 min, the trial was recorded as 'no interaction.'

In 2003, six trials were conducted using the six females and five males over 30-d (17 January–15 February 2003), for 16 d. Trials were conducted daily or every other day in 2003. In 2004, 10–12 trials were conducted daily to every other day, alternating between days with five control males vs. five control females and six experimental males vs. six experimental females, and the next day five control males vs. five experimental females and six control females vs. six experimental males. This method of pairing was used to insure that each lizard was used only once daily. Trials were conducted over a period of 30 d (13 January–11 February 2004), for 17 d. After each trial in 2003 and every fourth day in 2004, I recorded the color of each female with three photos (ventral, dorsal, and lateral) with a digital camera (Sony® TRV38, Tokyo). From each digital photo, ten locations with the brightest orange-red color were chosen and RYB color-saturation indices were recorded (following Summers and Clough, 2001) using Adobe Photoshop v. 6.0 (Adobe Systems, Mountain View, CA).

Videotapes were analyzed by recording the frequency and nature of the aggressive and receptive behaviors exhibited by both males and females.

#### *Color Manipulation: Male Choice*

This portion of the experiment tested whether males have a preference for females lacking or exhibiting breeding coloration. Color manipulation is used to isolate the role of behavioral signals from those of color. Similar studies have been conducted on other iguanian lizards: *H. propinqua* (Cooper et al., 1984), *Sceloporus virgatus* (Vinegar, 1972), and *H. maculata* (Hager, 2001) to examine the function of breeding coloration in these species.

Twelve plain female and four male leopard lizards were used in this experiment. Six females were painted using non-toxic decorative paint (mixture of red and orange Crayola<sup>®</sup> washable kid's paint, Easton, PA) to look like females with breeding coloration and six females were not painted (controls). The 2 x 4-m arena was set up as described above, but divided into three equal sections using Plexiglas<sup>®</sup> partitions. One painted and one plain female was introduced to either side of the arena and given ~60 s to become accustomed to the enclosure. A male was then placed in the middle section. The male was given 3 min to make a choice between the plain and painted female. Male behaviors were videotaped as described above. A choice was scored when the male ran up to the Plexiglas<sup>®</sup> and attempted to get to one side of the arena by pushing his body against or digging at the base of the divider. To test whether male choice was influenced by factors other than female coloration, males were given pairwise choices of two females, one painted and one unpainted, and then female coloration was reversed for these same lizards (unpainted female now painted, painted female now plain) and the same male was allowed to make his choice again. Thus, each female served as her own control. A total of 48 pairwise trials were conducted from 27 May–12 June 2003. More experiments will be conducted in the spring of 2004.

#### *Color Manipulation: Female Choice*

This portion of the experiment was conducted to determine whether females lacking or exhibiting breeding coloration behave differently toward other females lacking or exhibiting breeding coloration. It has been suggested that breeding coloration may serve as a social signal to conspecific females (Cooper and Greenberg, 1992). Preliminary data for *G. wislizenii* suggest

that females exhibiting breeding coloration are more tolerant of other females exhibiting breeding coloration than of females lacking breeding coloration (Moore, 1983).

Four females with natural breeding coloration (near peak brightness) and 11 females without breeding coloration were collected from Hawthorne Army Depot, Hawthorne, NV in early June 2003 for use in these experiments. The laboratory arena was divided into three sections as described above. Single females either exhibiting or lacking breeding coloration were placed on either side of the arena, and a focal (chooser) female was placed in the middle section. The behavior of the female in the middle was videotaped for 3 min and categorized in one of three ways: (1) flee, (2) ignore one or both females, (3) approach, display (pushups), and attempt to make contact (via scratching the Plexiglas<sup>®</sup> barrier) with either female. Choice for one of the two females was scored when the focal female ran up to the Plexiglas<sup>®</sup> and displayed or pressed up against or dug at the divider. Ten trials were conducted using females with breeding coloration as the choosers, and 15 trials using females lacking breeding coloration as the choosers. Trials for this portion of the experiment were conducted between 8–12 July 2003. More experiments will be conducted in the spring of 2004.

#### *Male Preference for Females during the Breeding Season: Lab*

This portion of the experiment will compare male behavior (aggressive and receptive) toward females exhibiting and lacking breeding coloration. Whereas the hormone implantation experiments were designed to monitor the change of male behavior as females developed breeding coloration, this portion of the experiment monitors the difference in male behavior towards females exhibiting a high intensity of breeding coloration and females lacking breeding

coloration. Furthermore, this portion of the experiment was conducted during the breeding season and thus female coloration was natural, rather than induced by use of hormones.

Each of 11 males was randomly introduced to the four females exhibited natural breeding coloration in the lab arena (as described above) and their behaviors were videotaped using a digital video camera for 3 min starting when either the male or female clearly acknowledged the presence of the other. Fifty-five pairings were conducted from 10 June–8 July 2003, 48 of which were conducted from 10–15 June 2003. Videotapes were analyzed by recording the frequency and nature of the aggressive and receptive behaviors exhibited by both males and females exhibiting or lacking breeding coloration.

#### *Male Preference for Females during the Breeding Season: Field*

In the field portion of this experiment, male preference for females with and without breeding coloration will be determined in nature. This portion of the experiment will be compared with experiments in the lab to determine whether behaviors are similar between lizards in a lab setting and those in the field.

Females will be introduced to males using a monofilament line from a 3-m fishing rod (Moore, 1983). Lizards will wear a harness made of fishing line and clear plastic so the female coloration is visible. When a male is located, the female will be slowly introduced ~0.5 m from the male. Interactions between the male and female will be videotaped for 3–5 min. One female with and one without breeding coloration will be introduced to each male for a total of 25 males.

### *Variance in Female Breeding Coloration*

I will document variation in color intensity among females in a single population (Hawthorne, NV) to determine the range and phenology of breeding coloration available to males throughout the breeding season. Females (N not determined) will be collected in the field. Mass and body length (SVL) will be recorded and digital photos (ventral, dorsal, and lateral) will be taken. Adobe Photoshop will be used to record the coloration of the lizards. From each picture, ten spots with the brightest orange/red color were chosen and their RYB scores will be recorded. Females will be released to their point of collection.

### *Monitor Hormone Levels in the Field*

May be included. Not yet determined.

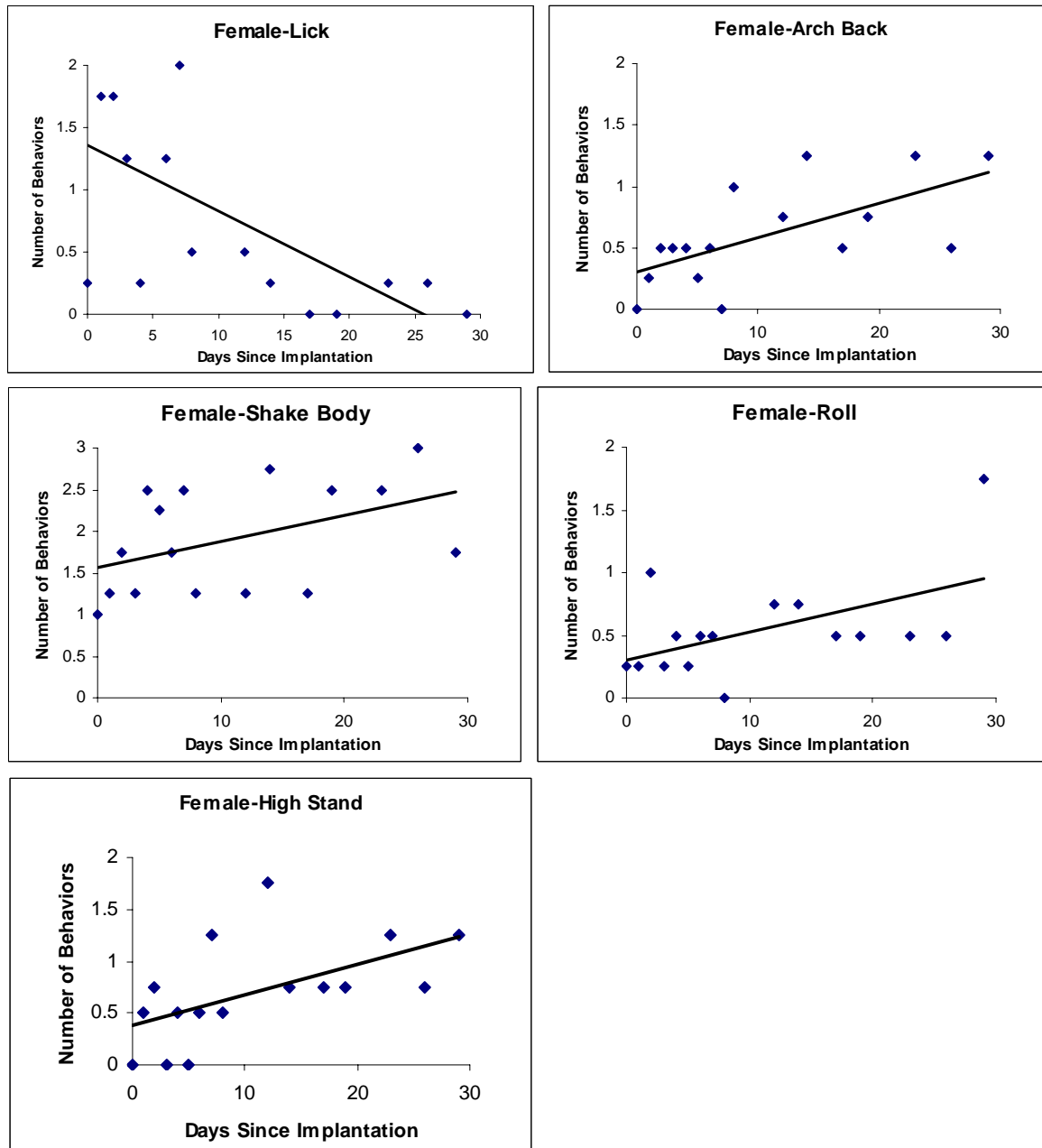
## **Preliminary Results**

### *Hormone Implantation*

There were 94 trials conducted over 30 d. I observed and recorded via videotape all behaviors that were associated with courtship. Fourteen behaviors associated with mating were performed by females and five behaviors associated with mating were performed by males (following definitions in Carpenter and Ferguson, 1977; Table 1).

Table 1. Courtship and rejection behavior exhibited by male (N = 5) and female (N = 5) *Gambelia wislizenii* implanted with hormones and the number of observations during the experiment.

Behavior	Sex Performing	Putative Role	Observation Frequency
Crawl over	Male	Courtship	305
Lick	Male	Courtship	178
First approach	Male	Courtship	77
Follow	Male	Courtship	51
Circle	Male	Courtship	131
First approach	Female	Courtship	10
Lick	Female	Courtship	50
Push-up	Female	?	53
Arch back	Female	Rejection	39
Shake body	Female	Rejection	122
Roll	Female	Rejection	35
High stand	Female	Rejection	45
Tail wave	Female	?	135
Sidlehop	Female	Rejection	47
Flight	Female	Rejection	25
Jerk side to side	Female	Rejection	16
Move from under male	Female	Rejection	40
Go away	Female	Rejection	55
Bow back	Female	Rejection	34



Figures 1–4: Frequency of behaviors exhibited as females developed breeding coloration. Lick, arch back, high stand, and roll were significant ( $P < 0.05$ ). Shake body was marginally significant ( $P < 0.01$ ).

Table 2: Summary table for linear regression of female behaviors.

Asterisks represent significant correlations ( $P < 0.05$ ) and

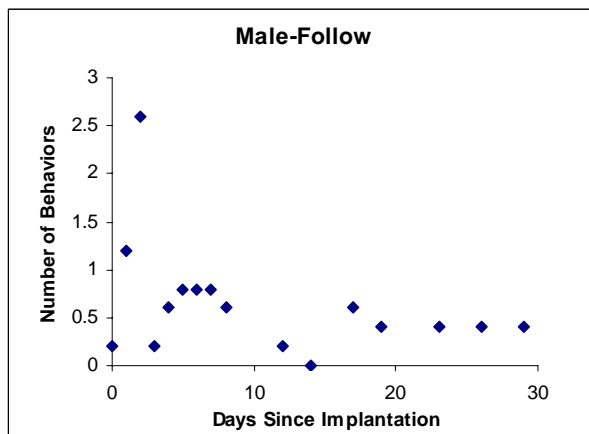
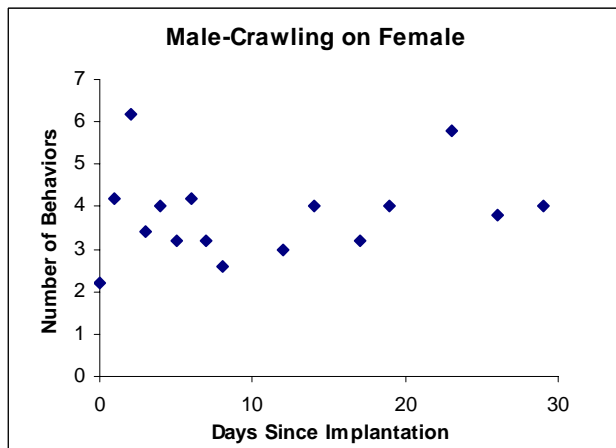
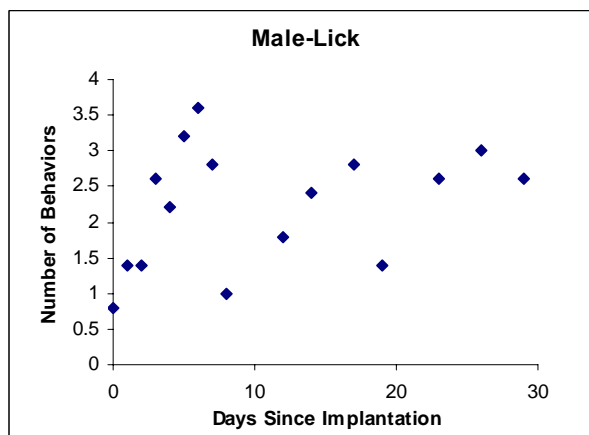
^ represent marginally significant correlations ( $P = 0.10-0.05$ ).

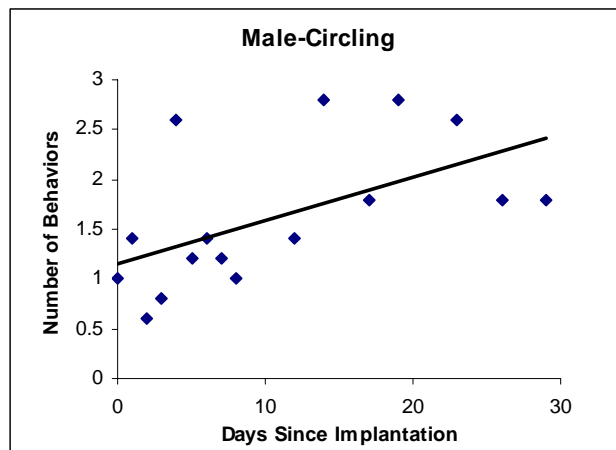
Behavior	$r^2$	$F$	$P$
First approach	0.328	1.691	0.214
Lick	0.625	8.955	0.010*
Pushup	0.392	2.535	0.134
Arch back	0.649	10.163	0.007*
Shake body	0.443	3.421	0.086^
Roll	0.523	5.260	0.038*
High-stand	0.565	6.569	0.023*
Tail wave	0.400	2.672	0.124
Sidle-hop	0.396	2.610	0.128
Flight	0.160	0.366	0.555
Jerk side to side	0.200	0.586	0.457
Move from under male	0.131	0.244	0.629
Go away	0.311	1.503	0.240
Bow back	0.018	0.005	0.947

The number of times each behavior was performed was recorded for each trial. Of the 19 behaviors observed, 14 female and five male behaviors were recorded with sufficient frequency to test for changes in these behaviors over time. Over a period of 30 d (16 trial days), the number of times aggressive behaviors were performed by females increased, whereas the number of receptive behaviors exhibited by females decreased (Figs. 1–4). This increase in female aggression was correlated with the development of breeding coloration. In contrast to the



females, males did not change the number of times they performed receptive behaviors over the same period (and as females developed breeding coloration). Males approached females in the same manner and frequency regardless of female coloration for four of the five most-common behaviors (Figs. 5–9). One male behavior, circling, increased significantly as females developed breeding coloration.





Figures 5–9: Male behaviors as females developed breeding coloration. N = 5. Significant results are represented by a line.

Table 3: Summary table for linear regression of female behaviors. Asterisks represent significant correlations ( $P < 0.05$ ).

Behavior	$r^2$	$F$	$P$
First approach	0.266	1.069	0.319
Lick	0.319	1.587	0.228
Crawl on female	0.185	0.495	0.493
Follow	0.382	2.388	0.145
Circle	0.562	6.464	0.023*

#### *Control Female*

Data not analyzed.

*Hormone Implantations-2004*

Data not analyzed.

*Female Coloration*

Data not analyzed.

*Color Manipulation: Male Choice*

Of the 42 trials (N = 4 males and 12 females) conducted for this experiment, males chose females painted to resemble females with breeding coloration 25 times and plain females 17 times ( $P > 0.10$ ). To test if males exhibited preferences for females independent of their coloration, males were presented the same females with and without breeding coloration (in random order). In these trials males chose the same female (once painted and once plain) eight times and a different female 10 times ( $P > 0.10$ ). More experiments will be conducted in the spring–summer of 2004.

*Color Manipulation: Female Choice*

Table 4. Matrix of color preference of females exhibiting and lacking breeding coloration. The chooser is the female that was given a choice between two females. The choices were introduced to the ‘chooser’ simultaneously.

		Chooser	
		Female (colored)	Female (plain)
Choice	Female (colored)	5	2
	Female (plain)	2	8

I used a Fisher's Exact Test to test whether females choose or were deterred by either colored or plain females. The choice by colored and plain females for colored or plain females was marginally significant ( $P = 0.058$ ). Yet, a sign test indicated no significance for colored females choosing plain vs. colored females, and plain females choosing plain vs. colored females. Moreover, observations indicated that females did not exhibit aggressive behaviors toward other females and females did not attempt to avoid each other (e.g., running away from the other female or crawling under a rock). More trials will be conducted during spring–summer of 2004.

*Male Preference for Female Exhibiting and Lacking Breeding Coloration During the Breeding*

*Season: Lab*

Data not analyzed.

*Male Preference for Female Exhibiting and Lacking Breeding Coloration During the Breeding*

*Season: Field*

No data collected.

*Summary*

My preliminary data (from hormone implantations and color-manipulations) suggest that female breeding coloration in *G. wislizenii* is not a signal to males, as previously reported for this species (Cooper and Greenberg, 1992) and for most other iguanian lizards (Cooper and Greenberg, 1992). Additional experiments will be conducted to confirm these findings. Future tests will also be conducted to understand whether breeding coloration is a signal to female

conspecifics. Understanding the behavioral aspect of breeding coloration will help us understand the role of this coloration and allow us to better understand how lizards communicate using visual signals.

### Literature Cited

- Andersson, M. 1994. Sexual Selection. Princeton Univ. Press, Princeton, NJ.
- Behler, J. L. and F. W. King. 1979. National Audubon Society Field Guide to North American Reptiles and Amphibians. Chanticleer Press, NY.
- Cooper, W. E., Jr. 1984. Female secondary sexual coloration and sex recognition in the keeled earless lizard, *Holbrookia propinqua*. *Animal Behaviour* 32:1142–1150.
- Cooper, W. E., Jr. 1986. Chromatic components of female secondary sexual coloration: influence on social behavior of male keeled earless lizards (*Holbrookia propinqua*). *Copeia* 1986:980–986.
- Cooper, W. E., Jr. 1988. Aggressive behavior and courtship rejection in brightly and plainly colored female keeled earless lizards (*Holbrookia propinqua*). *Ethology* 77:265–278.
- Cooper, W. E., Jr. and R. F. Clarke. 1983. Steroidal induction of female reproductive coloration in the keeled earless lizard, *Holbrookia propinqua*. *Herpetologica* 38:425–429.
- Cooper, W. E., Jr. and D. Crews. 1988. Sexual coloration, plasma concentrations of sex steroid hormones, and responses to courtship in the female keeled earless lizard (*Holbrookia propinqua*). *Hormones and Behaviour* 22:12–25.

- Cooper, W. E., Jr. and N. Greenberg. 1992. Reptilian coloration and behavior. Pp. 298–422, in: *Biology of the Reptilia*. Vol. 18 C. Gans and D. Crews (eds.), University of Chicago Press, Chicago, IL.
- Cooper, W. E., Jr. and J. A. McGuire. 1993. Progesterone induces bright orange throat coloration in female *Petrosaurus mearnsi*. *Amphibia-Reptilia* 14:213–221.
- Espinoza, R. E., C. R. Tracy, and C. R. Tracy. 1998. A safe, single-application procedure for eradicating mites on reptiles. *Herpetological Review* 29:35–36.
- Ferguson, G. W. 1976. Color change and reproductive cycling in female collared lizards (*Crotaphytus collaris*). *Copeia* 1976:491–494.
- Fox, S. F., J. K. McCoy, and T. A. Baird (eds.). 2003. *Lizard Social Behavior*. Johns Hopkins Univ. Press, Baltimore, MD.
- Hager, S. B. 2001. The role of nuptial coloration in female *Holbrookia maculata*: evidence for a dual signaling system. *Journal of Herpetology* 35:624–632.
- Mayhew, W. W. 1968. Biology of desert amphibians and reptiles. Pp. 195–356, in: *Desert Biology*. G. W. Brown (ed). Academic Press, New York.
- Medica, P. A., F. B. Turner, and D. D. Smith. 1973. Hormonal induction of color change in female leopard lizards, *Crotaphytus wislizenii*. *Copeia* 1973:658–661.
- Montanucci, R. R. 1965. Observations on the San Joaquin leopard lizard, *Crotaphytus wislizenii silus* Stejneger. *Herpetologica* 21:270-283.
- Montanucci, R. R. 1967. Further studies on leopard lizards, *Crotaphytus wislizenii*. *Herpetologica* 23:119–126.

- Moore, E. 1983. The function of orange breeding coloration in the social behavior of the long-nosed leopard lizard (*Gambelia wislizenii*). Unpublished Master's Thesis, Oregon State University, Corvallis, OR.
- Sugerman, R. A. 1980. Observer effects on collared lizards. *Journal of Herpetology* 14:188–190.
- Summers, K. and M. E. Clough. 2001. The evolution of coloration and toxicity in the poison frog family (Dendrobatidae). *Proceedings of the National Academy of Sciences* 98:6227–6232.
- Tollestrup, K. 1983. The social behavior of two species of closely related leopard lizards, *Gambelia silus* and *Gambelia wislizenii*. *Zeitschrift für Tierpsychologie* 62:307–320.
- Turner, F. B., J. R. Lannom, Jr. P. A. Medica, and G. A. Hoddenbach. 1969. Density and composition of fenced populations of leopard lizards *Crotaphytus wislizenii* in southern Nevada. *Herpetologica* 25:247–257.
- Vinegar, M. B. 1972. The function of breeding coloration in the lizard, *Sceloporus virgatus*. *Copeia* 1972:660–664.
- Watkins, G. G. 1997. Inter-sexual signaling and the functions of female coloration in the tropidurid lizard *Microlophus occipitalis*. *Animal Behaviour* 53:843–852.