Nesting and False Nesting Behaviors of Gopher Tortoises (Gopherus polyphemus)

Integrating all aspects of a species’ reproductive biology is important to any successful conservation management plan. Yet for many species of conservation concern, we lack complete descriptions and understanding of important behaviors, such as courtship, mating, nesting, egg laying, and hatching. This is true for nesting behaviors of the Gopher Tortoise (Gopherus polyphemus), a fossorial species native to the southeastern United States (Diemer 1986). Despite multiple studies of Gopher Tortoise movements, reproductive output, and mating behavior (McRae et al. 1981; Boglioli et al. 2003; Rothermel and Castellón 2014), to date, there are only two published accounts of nesting. One describes a single event by a field-collected tortoise housed in Connecticut (Kenefick 1954) and the second describes nesting behavior in a wild population of Gopher Tortoises in Georgia (Radzio et al. 2017a).

The lack of knowledge regarding Gopher Tortoise nesting behavior is troubling given their vital role as a keystone species (Guyer and Bailey 1993; Simberloff 1998) and their ongoing population declines (Mushinsky et al. 2006; U.S. Fish and Wildlife Service 2011). Gopher Tortoises are ecosystem engineers, digging large burrows used by over 300 other species (Jackson and Milstrey 1989; Lips 1991; Kinlaw and Grasmueck 2012; Catano and Stout 2015). In addition, the large mound of soil created during burrow excavation, called the apron, undergoes succession as plants colonize the bare sand, thereby increasing local plant diversity (Kaczor and Hartnett 1990). Loss of habitat and fire suppression pose major threats to remaining populations of Gopher Tortoises (Diemer 1986; Mushinsky et al. 2006). They require xeric habitats with sandy, well-drained soils for burrowing and nesting as well as sufficiently open upper canopy to support thermoregulation and abundant herbaceous forage (Douglas and Layne 1978; Diemer 1986; Nussear and Tuberville 2014).

Here, we describe nesting behaviors of wild Gopher Tortoises at Archbold Biological Station (ABS) in south-central Florida. We obtained video footage from 37 remote, infrared-triggered cameras, placed in front of and facing the mouth of the burrow so as to capture the burrow apron where nesting often occurs (Smith 1995; Butler and Hull 1996; Boglioli et al. 2003; Johnson et al. 2007; Lamb et al. 2013). We inventoried all potential nesting behaviors and conspecific interactions that occurred during each nesting event. Our goals were to: (1) determine if nesting behaviors in the field matched those previously reported (Kenefick 1954; Radzio et al. 2017a); (2) clarify the sequence and frequency of behaviors that lead to successful nesting; and (3) determine the potential reasons for failed nesting attempts.

**Materials and Methods**

The ABS is a 2102-ha Florida scrub preserve on the southern end of the Lake Wales Ridge in peninsular Florida. Within ABS, there is a particularly high density of Gopher Tortoises in Hill Garden (HG; 27.18699°N, 81.33854°W), a 4.5-ha old field embedded in a fire-dependent sandhill vegetation community (Ashton et al. 2008). Tortoises in and around HG are individually marked (by drilling of marginal scutes and numbers written on the carapace with a white paint pen) as part of an ongoing demographic study.

We collected behavioral data on 37 female tortoises in the HG site using Bushnell Nature View HD trail cameras (Model 119438) deployed at female-occupied burrows during the nesting season from March to June 2015. Each female was fitted with a glue-on radio transmitter (ATS model R1860), which enabled us to locate females every 3–5 days and move cameras for continued monitoring if they moved to or constructed a new burrow. Each camera had an infrared sensor that when triggered, recorded a 30-sec video, with a 3-sec lag between potential trigger events. We placed each camera so that it faced the burrow entrance with the widest field of view possible. Cameras were positioned pointing to the entrance of the burrow between 36 and 76 cm from the burrow mouth at a height ranging from 20–40 cm. We checked cameras and downloaded video files every 4–7 days.

We reviewed 956.3 h of footage, corresponding to an average of 182.7 ± 16.70 (mean ± 1 SD) h per female (range 107–201 h). From the footage, we identified 189 30-sec video segments containing nesting behaviors, totaling 01:34:30 h of nesting footage. We focused on those segments to identify finer-scale behaviors displayed by female tortoises. For each nesting event, we recorded the following data: (1) date, (2) time of day (reported in Eastern Standard Time), (3) event duration, (4) tortoise identity, (5) number of nesting attempts in the event, (6) time spent actively digging, (7) number of times the tortoise touched her snout to the ground, (8) number of head-bobs, (9) number of hindlimb thrusts into apron sand per minute, and (10) nesting event outcome. We followed the nesting behavior stages defined by Radzio et al. (2017a) and considered the onset of a nesting event when we observed behaviors including circling and digging a shallow depression, hindlimb digging of egg cavity, oviposition, covering the nest, and manicuring the nest area (Kenefick 1954; Radzio et al. 2017a). We defined the end of the event as the point when the tortoise ceased nesting behaviors and walked away from the nesting site and/or out of camera view for more than 5 min.

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Nesting event outcomes included (1) completion of a true nesting event, (2) incomplete nesting event due to the female aborting the nesting attempt, (3) incomplete nesting event due to interruption by a conspecific, and (4) incomplete sequence due to video footage interruption. True nesting events (outcome 1) were defined as behaviors associated with egg deposition or nest manicuring (Kenefick 1954; Radzio et al. 2017a) and confirmation of eggs present in the nest. To verify nesting outcome, from May to July 2015 we searched burrow aprons for nests constructed by telemetered females. We defined false nesting events (outcome 2) as cases in which the female was recorded completing behaviors up until digging the egg chamber, but we did not recover eggs from that apron (Ashton and Ashton 2008; Nelson et al. 2009). Unfortunately, we cannot differentiate all false nesting cases from interrupted nesting attempts (outcome 3) based solely on the video footage because our cameras may have missed interactions with conspecifics not on the burrow apron.

RESULTS

We observed 13 nesting events by seven females. Only one event (female 569) contained more than one nesting attempt (Table 1). Within this event, the two attempts were only 2 min apart, with the female briefly retreating into her burrow between attempts. Of the events observed, six were aborted by the female, one of which was aborted due to interruption by a conspecific male. A complete nesting event by female 1037 (on 24 April 2015) was captured on video, but for most others only a portion of the nesting event was recorded (Table 2). Notably, four female tortoises displayed nesting-related behaviors at burrows other than the burrows where they resided during April–May 2015.

In most cases, the observed nesting events followed the same behavioral sequence described in Radzio et al. (2017a). Most events began with females walking on to the burrow apron. Females then walked around the apron, using their forelimbs to dig up small amounts of sand. In some instances, females pushed soil with their forelimbs and body in a sliding motion (Fig. 1A). After pushing or digging the soil, they then spread out their forelimbs and planted themselves firmly in the ground (Fig. 1B) while facing away from the burrow, then dug using their hindlimbs. While digging, females swung the posterior end of the carapace with each leg thrust, swinging to the left when making a right leg thrust and vice versa. All the females dug in a rhythmic fashion, alternating legs after one to two thrusts.

The position of our cameras did not allow us to directly observe egg deposition. Thus, we could not confirm whether eggs

![Fig. 1. Images of specific nesting behaviors captured in videos of female Gopher Tortoises at Archbold Biological Station. A) Female 1037 raises her carapace out of the sand after having pushed herself into the ground. B) Female 569 braces her forelimbs before digging with her hindlimbs. C) Female 21 at burrow mouth and female 1037 circling and digging a shallow depression at the start of her event. D) Female 1037 midway through circling, with female 21 still in the burrow mouth covered with sand.](image-url)
were laid until we excavated every burrow apron between 1 May and 9 July. Female 1037 engaged in behaviors associated with egg deposition (Kenefick 1954; Ashton and Ashton 2008; Radzio et al. 2017a) at one burrow and egg cavity digging at two, but we did not find nests at either site (details below and in Table 2). In the five false nesting events, the female aborted the nesting attempt and walked away from the partially dug nest and back into the burrow (Table 2).

Although most nesting events followed the behavioral patterns described above, we also observed behaviors specific to particular females and events (Table 1). Behaviors specific to individual events included females touching their snouts to the ground and head bobbing. Below, we provide brief accounts of the 13 nesting events.

**Female 419.** We observed female 419 in an incomplete nesting sequence due to footage interruption on 5 May 2015 at 1453 h. The footage shows female 419 manipulating the nest site and begins with her walking over the burrow apron repeatedly, pushing soil with her carapace while touching her snout to the ground. Over 8:47 min she walked around the burrow apron, crossing it 15 times and touching her snout to the ground 43 times. She also pushed sand with her forelimbs and plastron as she moved across the apron. After moving around the apron pushing soil, she turned to face the burrow entrance and began walking toward the burrow, at which point the video footage was interrupted.

**Female 569.** We observed three false nesting events (Table 2). In each event, the footage shows female 569 digging the egg cavity while bobbing her head intermittently before and during the nest-digging process. In the first nesting event on 28 May at 1659 h, she walked out of the burrow, touching her snout to the ground five times at intervals of 1–3 sec. Once out of the burrow, she pushed soil in the apron directly in front of the burrow using her forelimbs and plastron. She then braced her forelimbs, facing away from the burrow entrance in the center of the apron. While in this position, she touched her snout to the ground four times in rapid succession (<1 sec intervals) and bobbed her head, paused, and then touched her snout to the ground once more, followed by a head bob. She then began digging with her hindlimbs. While digging, she paused a total of five times, during which she alternated being idle with short bursts of head bobbing. After 6:47 min of digging with pauses, she yawned, turned around, and walked back into the mouth of her burrow, ending the first nesting attempt. Then, 2:00 min later at the mouth of the burrow, she pushed soil with her forelimbs for 20 sec and then touched her snout to the ground three times in rapid succession. She then dug with her hindlimbs. While digging, she touched her snout to the ground nine times at irregular intervals. After digging for 2:50 min, the female paused, then bobbed her head twice in rapid succession before continuing to dig. After 20 more sec, she stopped digging, turned around, and retreated into the burrow, aborting the second nesting attempt.

In the second nesting event on 28 May at 1555 h, female 569 walked out of her burrow and touched her snout to the ground three times at 1-sec intervals. Once the female reached the center of the apron, she dug with her hindlimbs. While digging, she paused a total of eight times. The pauses came after bouts of digging that ranged from 13 sec to 2:00 min. The last pause continued for 17 sec, after which she quickly jerked her head into her carapace once, and then slowly began turning herself to face the burrow. She then slowly retreated into the burrow, aborting the nesting attempt.

The behaviors of female 569 during the third nesting event on 29 May at 1358 h were very similar to the first two events, although the third event was much shorter (Table 1). In this event, she turned to face away from the burrow and 12 sec later touched her snout to the ground twice in rapid succession. She then dug with her hindlimbs. While digging, she paused twice and head bobbed. Pauses occurred after bouts of digging ranging from 10 sec to 1:37 min. After the second pause she turned and slowly retreated into the burrow, aborting the nesting attempt.

**Female 663.** We observed part of a true nesting event on 10 May at 1659 h. The footage shows her digging the egg cavity. She dug with her hindlimbs, raising the anterior half of her plastron in the air while digging. Although we recorded only a single 30-sec clip of video from this event, we believe it resulted in a
Tortoise ID | Date       | Start time (hr:min:sec) | Event burrow | Nest in apron (Y/N) | Home burrow (Y/N) | Outcome
--- | ----------------- | ------------------------- | -------------- | ------------------- | ------------------- | -------------
419  | 5 May            | 1453 h                  | 0:25:51       | 750                 | N                  | Y             | Unknown
569  | 28 May           | 1130 h                  | 0:19:24       | 703                 | N                  | Y             | False nesting
569  | 28 May           | 1555 h                  | 0:23:18       | 703                 | N                  | Y             | False nesting
569  | 29 May           | 1358 h                  | 0:09:36       | 703                 | N                  | Y             | False nesting
563  | 10 May           | 1659 h                  | 2:19:35       | 699                 | Y                  | N             | True nesting
718  | 24 April         | 1050 h                  | 1:52:16       | 745                 | Y                  | N             | True nesting
1037 | 24 April         | 1053 h                  | 0:28:29       | 734                 | N                  | Y             | False nesting
1037 | 9 May            | 0946 h                  | 0:08:07       | 669                 | N                  | N             | Unknown
1037 | 11 May           | 1117 h                  | 0:32:40       | 669                 | N                  | Y             | False nesting
1037 | 13 May           | 1015 h                  | 0:05:02       | 752                 | N                  | Y             | Unknown
1037 | 16 May           | 0958 h                  | 0:42:01       | 660                 | N                  | N             | Unknown
1468 | 8 April          | 1028 h                  | 0:12:11       | 542                 | N                  | N             | Incomplete; interrupted by male
1680 | 26 April         | 1030 h                  | 3:44:04       | 565                 | Y                  | Y             | True nesting

### Table 2. Summary of 2015 nesting events and outcomes describing the tortoise involved, when and where the event occurred, whether a nest was found in the apron, and whether the nesting event occurred at the female’s home burrow. Unknown outcomes were due to insufficient or interrupted footage.

Female 718. We observed part of a true nesting event at 1050 h on 24 April. The footage shows female 718 nest manicuring. She walked over the apron, dug with her forelimbs while walking, and scanned left and right with her head. Throughout the event, she touched her snout to the ground a total of 22 times in bursts of 1–3. While manicuring, she walked into the burrow mouth four times for an average time of 10:28 min (range 3:06–16:25 min) and dug with her forelimbs while in the burrow, throwing sand out that had accumulated in the mouth. After 1:49 h of manicuring, female 718 walked into the burrow a final time, ending the event. Based on White et al.’s (2018) parentage assignment, the eggs subsequently found at this burrow (Table 2) belonged to female 718.

Female 1037. We observed one complete behavioral sequence, although we found no eggs in the apron and thus classified it as a false nesting event. We also observed one other false nesting event and three incomplete nesting events due to footage interruption. In the complete event on 24 April at 1053 h, she began by using her forelimbs and body to push copious amounts of soil in the apron for 06:37 min, until her carapace was half–buried in the ground, deeper than observed for other females. While pushing the soil, she touched her snout to the ground 22 times in bursts of 1–3. While manicuring, she walked to her left into the brush adjacent to the apron.

Female 1037 used her forelimbs to dig and push soil on the apron for 3:11 min while circling around her center axis three times before digging the egg cavity. While digging, she paused a total of nine times, during which she alternated between short bursts of head bobbing and touching her snout to the ground. The pauses came after bouts of digging that ranged from 5 sec to 1:16 min. After 32:40 min of activity, she stopped digging with her hindlimbs and crawled away from the apron and retreated into the burrow.

In the first incomplete nesting event on 9 May at 0946 h, the footage shows female 1037 circling and digging a depression likely midway through a nesting event. She touched her snout to the ground six times. She walked onto the burrow apron and walked toward the mouth of the burrow, digging with her front legs as she moved. When female 1037 reached the mouth of the burrow, she circled around her center axis on five occasions, moving between the burrow mouth and the apron briefly after each occasion for an average of 1:15 min (range 0:50–1:30 min) between each circling.

Female 1037 bobbed her head in bursts of two and touched her snout to ground after circling the first, third, and fourth time. After the fifth circle, she walked toward the center of the apron and walked to her left into the brush adjacent to the apron.

In the second incomplete nesting event on 13 May at 1015 h, the footage shows female 1037 circling. The portion of the nesting event we observed lasted 5:30 min. She sat at the mouth of the burrow and circled around her center axis twice over 3:00 min before walking out of the mouth on to the apron for 1:00 min. She then walked back to the mouth of the burrow, turned around, and walked off the burrow apron for a final time.

In the third incomplete nesting event on 16 May at 0958 h, the footage shows female 1037 circling and digging a depression in female 21’s burrow apron. Throughout the event female 21 was within the burrow mouth (Fig. 1C). Female 1037 circled around her center axis in the same location on the center of the apron on 10 occasions over a period of 40:40 min. During this time, she walked off the apron intermittently to the right before returning and circling again. Female 1037’s circling pushed sand to the sides of the apron, covering the burrow mouth with female 21 still inside (Fig. 1D). By her sixth time approaching the apron, the burrow mouth was completely covered and she had created a deep...
Nest construction behaviors of *G. polyphemus* are distinct from behaviors exhibited during burrow construction or burrow maintenance (Ashton and Ashton 2008). Digging with hindlimbs, bracing with forelimbs, and swinging the posterior end of the carapace side to side are all behaviors unique to nest construction. The purpose of all three of these behaviors seems to be digging a nest to the optimal depth. The bracing of the forelimbs likely provides the leverage necessary for the female to swing her carapace to accommodate leg thrusts. The swinging motion of the carapace assists the female in reaching as far into the ground as possible with each leg thrust while digging and rounding out the shape of the cavity (Kenefick 1954; Radzio et al. 2017a).

Because of the camera angle in our study, we were unable to observe actual egg deposition, which was the final step of nesting behavior described by Kenefick (1954) and the third stage described by Radzio et al. (2017a). However, we observed all of the other behaviors described by Radzio et al. (2017a), including circling and digging a shallow depression, digging the egg cavity, nest covering, and nest manuering. Thus, our observations are generally consistent with those previously described.

Gopher Tortoises often touch the ground with their snout during nesting. Radzio et al. (2017a) noted ground nuzzling only as a post-nesting behavior, and speculated that this nuzzling was done to hide olfactory cues of nesting from predators. This differs from our observations, in which tortoises touched their snout to the ground throughout the nesting sequence. We hypothesize that this snout touching could have multiple roles that vary depending on when it is performed during nesting. The first is that this behavior could be a means of sensing soil composition, temperature, and moisture levels before nesting begins. Adequate soil moisture is necessary for a nest cavity to hold its structure and not fall apart during excavation and also prevents egg desiccation during incubation (Ashton and Ashton 2008). However, high soil moisture or clay content can inhibit hatchling emergence (Brode 1959; Epperson and Heise 2003). Ground nuzzling is also used for site selection in other turtle species (Morjan and Valenzuela 2001). A second possible function of this behavior is to determine burrow ownership based on chemical signals or scents before nesting and to establish burrow ownership following nesting. Tortoises in the genus *Gopherus* use pheromones from mental glands on the chin, as well as scat, to signal to conspecifics (Winokur and Legler 1975; Auffenberg 1977; Alberts et al. 1994). Females of the closely related Desert Tortoise (*Gopherus agassizii*) often touch their snout to the ground at burrow entrances to detect the scent of feces from other females during the nesting season (Ruby and Niblick 1994; Bulova 1997). Determining if a burrow is occupied by another tortoise may be valuable information for nesting females, because female-female aggressive encounters, sometimes involving combat, do occur (Weaver 1970; Douglass 1986; Radzio et al. 2016).

We determined that only 2 out of the 14 nesting attempts resulted in nests at the site where we recorded the female behaviors. Eleven events were incomplete behavioral sequences, because females aborted nesting, were interrupted, or our video footage was discontinuous. In one nesting event with a complete behavioral sequence (Female 1037), the female exhibited behaviors associated with nest digging, oviposition, and nest covering, but we did not find this nest. This was likely an instance of false nesting although we cannot rule out the possibility the nest was depredated. In most instances of false nesting, the female aborted the attempt after a few minutes of digging, leaving behind a partially dug-out cavity. False nesting is common in Gopher Tortoises (Butler and Hull 1996; Ashton and Ashton 2008; Lamb et al. 2013) and many other turtle species (Ivanchev 2007; Nelson et al. 2009) and may reflect unsuitable abiotic conditions (e.g., too dry, hot, or shaded) or efforts to distract predators from real nests (Ivanchev 2007; Lamb et al. 2013). Alternatively, females may have been disrupted by unseen humans or conspecifics out of view of the camera, especially in cases where females paused digging activity and engaged in head-bobbing (Females 568 and 1037).

Motion-triggered cameras efficiently captured large-scale patterns of burrow use in our wild population, yet our study revealed some potential limitations of these cameras for studies of tortoise behavior. First, our camera position was too low to the ground to document behaviors such as egg deposition within the constructed nest. To better view such behaviors in future studies, we recommend a raised camera angled down toward the burrow apron. Also, the cameras we used were triggered by temperature changes in the camera’s field of view, such as the body heat of an organism. However, due to their ectothermic nature, detection of tortoise movement may have been hindered by the limited difference between a tortoise’s body temperature and the environmental background. Limited detection of ectotherms by passive
infrared triggering systems has been reported previously in other studies (Somaweera et al. 2011; Welbourne 2013; Meek et al. 2015). We cannot know how many events or behaviors we missed as a result of poor thermal detection.

In addition to documenting false nesting behaviors (Lamb et al. 2013), our study also indicates individual variation in tortoise nesting activity, including behaviors suggestive of nest-site selection. We observed females constructing nests at their own burrow aprons and on the aprons of burrows that they did not reside in, including those occupied by another tortoise (Female 1037 during two events; Table 2). Female Gopher Tortoises in Georgia have also been observed nesting at other females’ burrows (Radzio et al. 2017a, b) and previous studies have documented Gopher Tortoise nests at juvenile tortoise burrows where the occupant was too small to be a reproductively mature animal (Ennen et al. 2009; Lamb et al. 2013; Radzio et al. 2017b). Thus, any study of reproduction in Gopher Tortoises will need to monitor all surrounding burrows for nests, rather than just those regularly inhabited by a focal female. Understanding how demographic (e.g., tortoise density, sex ratios; Gayer et al. 2012), social (e.g., interspecific interactions; Radzio et al. 2016, 2017a), and environmental factors potentially contribute to nest-site selection and false nesting events may be important for determining influences on the spatial distribution of tortoise nests in natural populations.

Acknowledgments.—We thank J. Sanchez and A. Haigh for their generous help reviewing video footage for tortoise behaviors and M. Stella for assistance in calculating total hours of footage. Field data collection would not have been possible without the assistance of M. T. White and E. Noel. Disney Conservation Fund, Gopher Tortoise Council, Riverbanks Zoo and Garden Conservation Support Fund, and American Wildlife Conservation Foundation provided funding. Support for KNW and TDT was provided in part by Award Number DE-FC09-07SR22506 from the U.S. Department of Energy to the University of Georgia Research Foundation. Research methods complied with Florida Fish and Wildlife Conservation Commission scientific collecting permits (LSSC-10-00043) and protocols approved by the University of Georgia Institutional Animal Care and Use Committee (A2014 05-024-Y3-A3).

Literature Cited


Sexual Dichromatism of Dorsal Stripes in “Red-back” Morph Individuals in a Population of Eastern Red-backed Salamanders (Plethodon cinereus)

In animals, coloration can differ between sexes, a condition termed sexual dichromatism. In amphibians, sexual dichromatism appears to have been understudied in comparison to other taxa (e.g., birds, fish, lepidopterans); however, it has been demonstrated to be present in anurans (e.g., Litoria lesueurii, Rana arvalis, Rhinella ictericus, and Lithobates sylvaticus; Bell and Zamudio 2012; Lambert et al. 2017). In salamanders, males often court females during mating and the males of some taxa (e.g., Salamandrella keyserlingii, Triturus sp.) are visually brightly-colored in certain body regions relative to females, suggesting a role of coloration in courtship (Salthé 1967; Hasumi 2001). Recently, a number of studies utilizing techniques such as spectrophotometry or digital image analysis have demonstrated previously undocumented sexual differences in the patterning and/or coloration of salamanders, such as Ambystoma maculatum, A. opacum, Notophthalmus viridescens, and Salamandra salamandra, suggesting sexual dichromatism may be more common in Caudata than previously realized (Todd and Davis 2007; Davis and Grayson 2008; Morgan et al. 2014; Balogova and Uhrin 2015).

Eastern Red-backed Salamanders (Plethodon cinereus) are small, relatively common terrestrial salamanders found in forest habitats throughout their range in eastern North America (Petranka 1998). This species exhibits a wide variety of color variation; at least eight distinct color phenotypes are present in this species (Moore and Ouellet 2014). The most common color phenotypes of Eastern Red-backed Salamanders are “red-back” and “lead-back” color morphs (Petranka 1998; Moore and Ouellet 2014). In both morphs, individuals typically bear a “salt and pepper” patterned venter and gray-black dorsolateral body; however, “red-back” morphs display a conspicuous dorsal stripe which is typically red-orange in coloration (Petranka 1998; Moore and Ouellet 2014).

Plethodon cinereus exhibiting the red-back morph can display considerable intra-specific variation in the redness of the dorsal stripe (Fig. 1); however, no previous studies appear to have examined this variation quantitively. Because recent studies have elucidated previously unknown sexual dichromatism in salamanders, this variation might be explained by sexual differences in the red coloration of the dorsal stripe in P. cinereus exhibiting the red-back morph. Thus, the objective of the present study was to utilize digital image analysis to quantify the coloration of the dorsal stripe of the red-back morph of P. cinereus to test for sexual dichromatism of the red dorsal stripe in red-back morph individuals of this species.

**Materials and Methods**

Plethodon cinereus were captured, photographed, and released on 29 April 2018 at a single site in Montour County, Pennsylvania, USA (41.0014°N, 76.7077°W; WGS 84). The study site consisted of an approximately 1-ha plot within a mixed-deciduous forest bordered by old field habitat. Salamanders were captured by hand underneath rocks, logs, and leaf litter. Salamanders were photographed and released on-site. Dorsal photographs of salamanders were taken within an enclosed 21 cm × 14 cm (5.5 cm...