Protocol

Captive Care, Raising, and Breeding of the Veiled Chameleon (*Chamaeleo calyptratus*)

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Squamate reptiles comprise approximately one-third of all living amniotes. In most of these species, it is difficult to study gastrulation and neurulation because the embryos are at a late stage of development at the time of oviposition. This is not the case, however, in veiled chameleons (*Chamaeleo calyptratus*), which are increasingly being used as a model organism to study these and other developmental and evolutionary phenomena. Originating from the Arabian Peninsula, veiled chameleons are arboreal specialists that possess extensive morphological specializations for climbing. They naturally inhabit semitropical habitats, but they also have an almost 30-yr history of being bred in captivity. Veiled chameleons breed readily and do not require a period of cooling to induce the reproductive cycle, and females can produce ~45–90 eggs multiple times per year. Thus, compared with other reptiles, relatively few animals are needed to maintain a productive breeding colony. Herein, we present the conditions, equipment, and techniques required for proper husbandry and breeding of veiled chameleons within a laboratory environment.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Bleach (use diluted 1/10 in water)
Calcium without vitamin D3 powder (phosphorous-free; Rep-Cal)
Calcium with vitamin D3 powder (phosphorous-free; Rep-Cal)
Chameleons (males and females; e.g., from FL Chams or LLL Reptile) (see Step 8)
Cricket gel water (or sponge saturated in water) for drinking
Fluker's high-calcium cricket feed
Food

We use crickets, as well as dandelions, collards, kale, carrots, and sweet potato.

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Herptivite multivitamin powder (Rep-Cal)
Soap
Vermiculite, for incubation medium
Water
Weeping fig (*Ficus benjamina*) or a large artificial plant (see Step 3)

Equipment

Aquarium, 10-gallon (with sliding screen lid)
This can serve two adjacent cages.

Blue daylight incandescent bulb (60 W or 100 W; Zoo Med)
Bucket (5 gallons) or plastic nursery pot (height: ~12 inches; diameter: ~10–12 inches) or plastic tub (~14 gallons)
Corrugated plastic sheets (nonpermeable), to visually separate chameleons
Decorative reptile plants, plastic or live, sufficient for a 10-gallon aquarium (LLL Reptile)
Deli cups or plastic Tupperware (with lids, no holes punched; TSK Supply)
Fluorescent hood fixture, large enough for a 48-inch light
This can serve two adjacent cages.

Handheld sprayer (1–2 gallons; source from hardware/garden store)
Incubator (e.g., Hova-bator egg incubator), set at 26°C–30°C
Paper towels, crumpled
Reflector dome, with ceramic light socket
Repti-Glo 5.0 UVB fluorescent light, 48 inches (Exo-Terra)
This can serve two adjacent cages.

Repti-Glo 5.0 UVB 26 W compact fluorescent bulb (Exo-Terra)
Room humidifier (optional)
Room space heater, with thermostat (optional)
Screen cages, for adults (24 × 24 × 48 inch [length × width × height] [e.g., from LLL Reptile])
Screen cage water trays [24 × 24 (length × width; LLL Reptile)]
Timer, for lights (12 h on:12 h off)
Tunnel materials
We use sand, organic fertilizer-free top soil, and fertilizer-free peat moss.

Water bowl, for incubator
Note that not all incubators require a source of additional humidity.

Water misting system (Mist King; optional, see Step 7)

METHOD

Initial Setup of a Colony

1. Maintain study rooms at a temperature of 21°C–24°C during the day, with 50% humidity, using a room humidifier if necessary.

   We use day:night cycles of 12:12 h (a night time temperature drop to ~12°C–15°C is acceptable, and use of a room space heater is optional).

2. Construct adult screen cages following the manufacturer's instructions. Mount the cages on top of water-retaining bases (screen cage water trays). Place two cages next to each other. Place a corrugated plastic sheet (cut to fit) between cages to inhibit visibility and minimize stress associated with territoriality.

3. Place a weeping fig or artificial plant into each adult cage.

   Robust perches such as artificial vines, branches, twigs, and Ficus *tree branches are required owing to the large size of* Chamaeleo calyptratus *as adults. Plastic is highly recommended in the laboratory setting to avoid porous plants and soil that harbor microbes or viruses. Extra decorative reptile plastic plants and vines are beneficial to chameleons for additional climbing opportunities, increased surface area for water to collect, exercise for autopodia, and provision of more hiding areas.*

4. Place a ceramic reflector dome on the top of each cage, ~4–8 inches above the highest basking perch inside the cage. Attach a 60–100 W blue daylight incandescent bulb (UVA).

   This serves as the heat source for proper body thermoregulation

5. Place a fluorescent hood fixture with an associated 48-inch Repti-Glo 5.0 UVB fluorescent light across the top of two adjacent cages.

6. Connect light sources to timer for a 12:12 h day:night cycle.

   Note that, although we use UVA and UVB light sources separately (Fig. 1A), mercury-based bulbs, which are available through reptile suppliers, emit both wavelengths simultaneously along with heat.

7. Establish a suitable watering system and schedule.

   - For large colonies (e.g., less than eight individuals), set up an automated watering system (e.g., Mist King). Secure the pipes across the top of the cages, and strategically place the spray nozzles over leaves or broad surfaces, where chameleons can drink from water droplets collecting and moving over such surfaces. Set the sprayer to "rain" a minimum of two to three times per day for 1–3 min per session.

     Chameleons drink from moving and amalgamated droplets on plants (not from water bowls) but also flare their lips laterally to ingest water flowing down the lateral margins of their snouts.

   - For small colonies (e.g., less than eight individuals), manually spray with a handheld sprayer two to three times per day for ~1 min per cage during the morning, midday, and evening.

     We prefer manual spraying to directly observe drinking and eyelid blinking (removal of debris trapped in the small eyelid opening). However, this strategy is time intensive for colonies greater than eight individuals.

8. Start a colony with juveniles (age 3–4 mo; three males and six females). Maintain two juveniles or one adult in each cage. For each cage with a female, fill a 5-gallon bucket, plastic nursery plant pot,

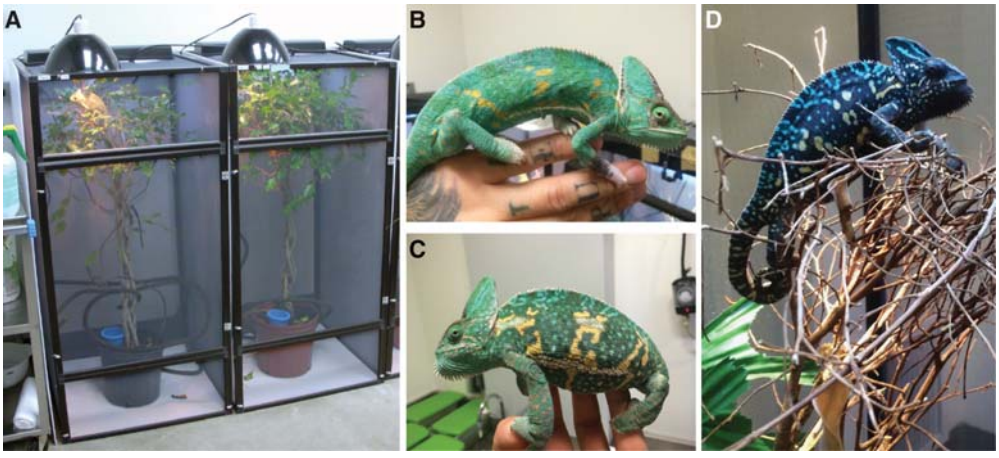

FIGURE 1. (*A*) Chameleon cages and cage setup. Screening provides ventilation. Ambient lighting is provided by a fluorescent UVB bulb and an incandescent UVA bulb for thermoregulation and for light. Vegetation provides perches and shelter. The bare floor is easy to clean. An automatic misting system provides drinking water and humidity. (*B*) A sexually receptive female displaying "robin blue" dorsal spotting on a pale, lime-green skin background color. (*C*) A recently mated female veiled chameleon *C. calyptratus* darkens the green background coloration and increases the depth of yellow on her lateral spots—this is frequently shown by animals in our laboratory postcopulation. (*D*) A nonreceptive female displays high-contrast blue-green and yellow spots on a high melanic background. This is generally presented in combination with mouth gaping and biting displays toward the male.

or 14-gallon plastic tub with 8 inches of tunnel material (a 1:1:1 mixture of peat moss, sand, and top soil), place it into the cage (Fig. 2B), and let it remain in the cage at all times as the female nears sexual maturity (~6 mo of age).

Males have an enlarged tarsal spur at hatching (Fig. 2E); during maturation, males also develop a larger overall size, an enlarged cranial casque, and a more vibrant coloration and patterning, including distinct vertical color bars on their flanks (Fig. 2A). Sexual maturity in C. calyptratus *is acquired between 4 and 6 mo posthatching. We breed female chameleons at ~8 to 9 mo of age, when growth has ceased (see Step 11); the females will use the tunnel material for egg-laying (see Step 15). The soil mixture must be moist enough to maintain tunnels without collapsing during digging, but not so wet that any water forms puddles.*

Ongoing Maintenance and Husbandry

9. Regularly perform both minor and major cleaning.

   i. On a daily basis, spot-clean as necessary, removing fecal matter, dead crickets, and leaves.

      The chameleons can remain in the enclosure during this process.

   ii. Once every 1–2 wk, wash the plastic base of the cage with soap and water, and disinfect using a 1:10 bleach solution.

      While major cage maintenance such as this is performed, remove the chameleon from the cage, and place it into a 10-gallon aquarium or opaque plastic tub with crumpled paper towels or other objects (for grasping).

   iii. Once every 2–3 mo, wash all plastic plants and décor with soap and water.

      As chameleons are greatly stressed by major rearrangements, only minimal changes are recommended.

10. Feed the chameleons crickets and plants appropriate for their body size at a schedule consistent with their growth and reproductive state. Maintain feeder crickets with cricket gel water and Fluker's high-calcium cricket feed. When feeding them to the chameleons, dust them with Herptivite multivitamin powder and/or mineral supplements.

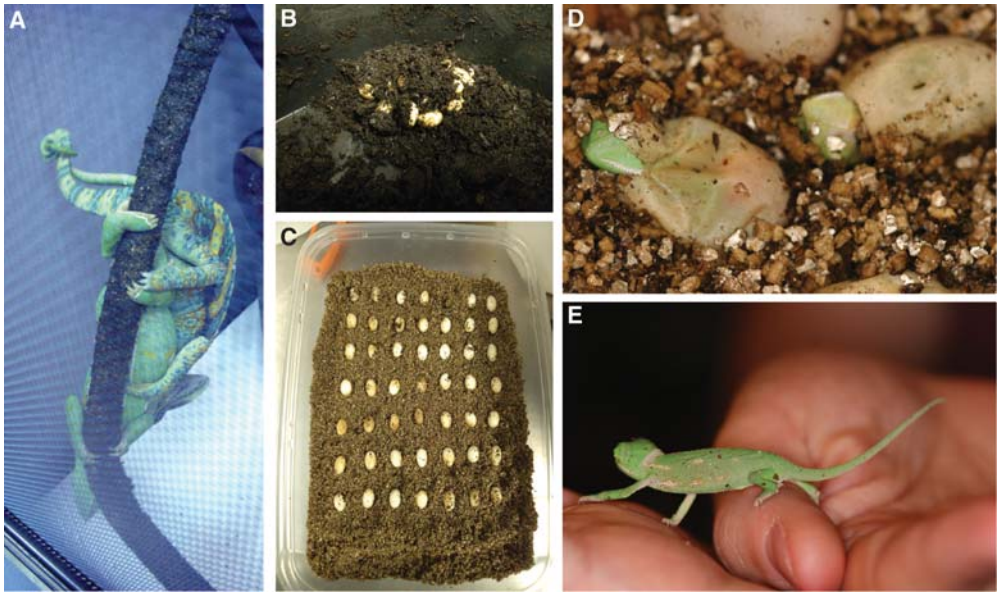

FIGURE 2. (*A*) Female veiled chameleons (*C. calyptratus*) are approached aggressively by males when introduced into their cage; this male is mating while both are on a vine. Males stroke the flanks of females with their tarsal spurs. (*B*) Egg clutches are deposited at the bottom of the provided substrate. (*C*) Eggs from *B* are sorted on moistened vermiculite before incubation. (*D*) Liquid seeps through the eggshell as the membranes increase in permeability. Chameleons begin to hatch at ~200 d into incubation at 26°C. (*E*) A neonate male with limited patterning and coloration (mainly lime green) and showing the sexually dimorphic tarsal spurs on the rear legs.

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i. Feed neonate and juvenile chameleons daily. Give them constant access to food. Dust feeders of neonate and juvenile chameleons with calcium powder without vitamin D3 two to three times per week, with calcium powder with D3 once to twice per week, and with vitamin powder once every 2 wk.

ii. Feed adults every second to third day. Dust their feeders with calcium powder without D3 one to two times per week, with calcium powder with D3 once per week, and with vitamin powder once every 3–4 wk. Give gravid females more frequent calcium dusting with D3.

Adult chameleons require ~8–10 appropriately sized crickets per feeding; adult females must not be over fed or allowed to become obese as this can result in dystocia (i.e., inability to oviposit eggs owing to oviductal obstruction) and other adverse health effects.

Feeder crickets must not be longer than the head of the chameleon is wide. As a gauge of size, we use 2-wk-old (1/8–1/4 inch) crickets for hatchlings, and two-thirds-grown crickets (~ 2/3 inch) for adults.

Sexing and Breeding

11. Introduce females into the enclosure of the male when blue spots are visible.

Females undergo constant cycling of clutches (Kummrow et al. 2010a,b) and display pale-blue ("robin's egg") spots on the dorsum, indicating receptivity for mating (Fig. 1B), which correlates with hormonal reproductive status.

12. Monitor the behavior of the chameleons on introduction for indications as to whether mating will occur (Fig. 2A), and do not leave paired chameleons together for >8 h.

Male courtship entails the exhibition of bright coloration, lateral body flattening, body swaying, and tail curling; the males approach females and nudge them with their chin while producing an internally generated vibratory stimulation during contact with the female—the female is mounted and the male uses its tarsal spurs to caress the flank of the female (see online Movie 1 at cshprotocols.cshlp.org). Copulation can last from a few minutes to an hour and can occur several times throughout the day. Females darken their green background coloration and increase the intensity of their yellow patterns on successfully copulating (Fig. 1C).

If a female is not receptive, her body coloration will become a dark brown to black, together with white or yellow mottled patterns (Fig. 1D); she might also become aggressive and bite the male if his advancements continue.

13. If necessary, reintroduce a female to the same male to secure a mating event.

3–7 d is a good waiting period before reintroducing a female into the male's cage.

14. Record in an Excel spreadsheet or other database the health, husbandry, breeding, and source information of the chameleons being studied.

Egg-Laying and Incubation

15. Monitor the female and check the soil (tunnel) material at the bottom of her cage until she lays her eggs.

Female chameleons decrease or cease feeding 3–4 wk after mating and spend more time at the bottom of the cage as they search for an appropriate egg-laying site. Females with soil or sand on their heads indicate that they have been digging. Tunnels will reach the bottom of the container and will be the site of egg deposition (see online Movie 2 at cshprotocols.cshlp.org). Tunnels are covered with substrate after oviposition is complete and are difficult to locate. Females might return to the tub within a week to dig another tunnel and lay additional eggs.

16. Give females plenty of water and crickets supplemented with calcium to aid in recovery after laying.

17. Provide a branch or rope extending into the egg-laying site from a higher source to allow females to freely climb in and out.

18. Prepare incubation medium by combining a 1:1 mixture (by mass) of vermiculite and water.

19. Carefully dig the eggs from the nest substrate, gently rub any soil substrate from the egg surfaces, and then place the eggs into deli cups or plastic Tupperware together with incubation medium (Fig. 2C). For optimal incubation, use a depth of 1–1.5 inches, with eggs buried lengthwise, half-covered by vermiculite. Do not rotate eggs during the transfer from the egg-laying site to

incubation tub. Keep a 0.5 to 1 inch space between neighboring eggs to allow for the eggs to grow or expand during incubation as they absorb moisture.

Unlike avian eggs, squamate embryos lack chalazae and are not suspended centrally in the egg. Instead, squamate embryos and extraembryonic membranes are in contact with the inner surface of the eggshell and will become damaged through egg rotation as this leads to internal shifting of yolk and membranes, ultimately leading to embryonic lethality by separating membranes from the eggshell. (Chalazae in avian lineages function to suspend the embryo and yolk centrally in the egg and always reorient the embryo to rest on the surface of the yolk after egg rotation.)

20. Incubate the eggs in the dark in a Hova-bator incubator at the desired temperature (26°C–30°C). Check the containers approximately weekly to measure moisture levels as well as looking out for ventilation mold growth; consider placing a water bowl within the incubator to increase the humidity of the chamber to 95%.

    The eggs will reach 1.5–2 times their size at oviposition upon continual absorption of moisture over the following several weeks of incubation. When eggs are near hatching, the eggshell and associated membranes appear to thin and allow for penetrance of fluids through the eggshell, giving the appearance that the eggs are "sweating."

21. Upon hatching (by ~200 d at 26°C), allow the neonates to roam the container for 1 d (enough time to internalize any remaining external yolk sac) and then move them into a nursery (Fig. 2D, E; see Step 22).

Rearing Young

22. Arrange a nursery to consist of a 10-gallon screen-top aquarium, with a base one-third to one-half filled with decorative plastic reptile plants. Place a Repti-Glo 5.0 UVB 26 W compact fluorescent bulb in a hood placed at one end of the aquarium to maintain the temperature in the range 21°C–28°C and to provide UVB. Provide a layout affording different plant heights below the bulb.

    The plants serve as hiding refugia as well as providing an increased surface area for water to collect after spraying for drinking and humidity. Using different plant heights allows different basking temperatures within the aquarium for proper thermoregulation.

23. Raise two to six hatchlings per aquarium for the first few months.

24. Move two individuals into an adult screen cage enclosure (with increased amount of plastic and real vegetation for hiding) when they reach a total length of 4–6 in.

25. Place chameleons in individual adult enclosures at an age of 4–6 mo.

DISCUSSION

Over two decades of captive husbandry of *C. calyptratus* has produced robust and tolerant veiled chameleons that in turn are easy to breed and maintain. Similar success has been attained for captive husbandry of various other chameleon species (de Vosjoli and Ferguson 1995; Schmidt 2001; Nečas 2004; Nečas and Schmidt 2004). In the laboratory, male–female pairing does not always lead to successful courting and copulation, and so it is advisable to have at least three sexually mature males available and four or more females for optimal combinations to elicit successful matings.

Veiled chameleons develop a broad array of health conditions in captivity, including hypovitaminosis A, hypervitaminosis A, renal failure, dystocia (egg binding—or a failure to lay eggs—which is generally fatal if not treated early through a caesarean section or spay surgery), respiratory problems owing to improper humidity or temperature, parasitic infections, and conditions involving the oral cavity (such as accidental introduction of substrate from a potted plant into the oral cavity that can become stuck in the oral surface, leading to infection) (Stahl 2006). An overabundance of UVB has recently been shown to cause corneal damage (which is reversible and not common unless the day–night hours are ignored) (Gardiner et al. 2009). The reader is encouraged to view several recent studies

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elucidating the important relationship between sufficient UVB lighting in the terrarium and skeletal homeostasis (Hoby et al. 2010, 2011; Haxhiu et al. 2014). In addition, excessive inbreeding can lead to congenital anomalies.

The problem of dystocia is paramount, as females that fail to find a suitable location to deposit their eggs will retain them, and subsequent egg growth renders the chameleon unable to pass the eggs through the oviducts and cloaca. Owing to the significant increase in clutch size of captive chameleons relative to wild chameleons in their native Middle East (~20 in the wild compared with 50–90 of captive animals [Schmidt 2001]), females can benefit from a decreased amount of food intake on reaching sexual maturity, which might limit not only the size of the eggs (i.e., increased yolking) but also their quantity. This issue needs to be explored in the future to further the responsible practice of captive breeding of the veiled chameleon.

For a discussion of *C. calyptratus* as an Emerging Model Organism for research, see **The Veiled Chameleon (*Chamaeleo calyptratus* Duméril and Duméril 1851): A Model for Studying Reptile Body Plan Development and Evolution** (Diaz et al. 2015).

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