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Parthenogenetic reptiles: New subjects for laboratory research

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Summary. Problems preventing establishment of laboratory colonies of parthenogenetic lizards have been solved. Now, productive colonies of these lizards, which have remarkably little genetic variation, can be readily established and used not only for research on parthenogenesis but also for many kinds of experiments for which reptile systems are desirable. Research colonies can provide valuable specimens while reducing the exploitation of natural populations.

An ideal laboratory animal for a wide variety of research is a small terrestrial vertebrate that is relatively easily maintained and bred, and which exhibits little genetic variation. Among the amniotes, unisexual (all-female) species of reptiles are among the best potential resources for such research, as well as for investigations of phenomena relating to parthenogenesis. These reptiles are the only vertebrates known to reproduce normally by true parthenogenesis[2]. Compared to gonochoristic (bisexual) species, the limited morphological variation in local population samples[3,4], the extensive histocompatibility between individuals[5-7], and the cytological mechanism by which they produce eggs[8,9] indicate that lineages of parthenogenetic lizards have remarkably little genetic variation. It may be that siblings normally are essentially genetically identical to each other and to their mother.

Until now, the major obstacle to using any of the approximately 27 species of parthenogenetic reptiles extensively for experimental work has been the inability to raise hatchlings to maturity with consistent reliability. There are only two reports[10,11], both recording extremely limited success, of raising hatchlings of parthenogenetic lizards to adulthood in isolation from males and then obtaining developing eggs (most of which spoiled) or additional hatchlings (a total of fewer than 10). Indeed, none of the approximately 6000 species of reptiles is yet being maintained and bred in laboratory colonies to produce experimental animals efficiently. Most biologists using reptiles must rely on purchasing animals that were harvested from natural populations. Such animals usually are of unknown background and often are in poor health, which may affect results of the experiments. In addition, this practice contributes to disruption of natural populations and is becoming increasingly difficult to rely on, particularly as more and more states and nations adopt necessary legislation to protect their wildlife. These problems now can be avoided by establishing laboratory colonies of parthenogenetic lizards. In addition, parthenogenetic species are maintained relatively efficiently; they do not require large territories and coincidental cycling of the reproductive systems of both sexes, and they have a high reproductive potential as all adults can produce offspring independently.

We detail here the system we have used to raise parthenogenetic lizards (Cnemidophorus exsanguis Lowe, 1956; a triploid species) through 3 generations (figure 1). This species, the Chihuahua whiptail lizard, has not been raised in captivity before. Thus, this also is the first demonstration that females of this unisexual species can reproduce after being hatched and maintained in captivity in complete isolation from males. Our procedures also should prove useful with gonochoristic species that are difficult or 'impossible' to raise.

Housing. About 6 adults or 15 hatchlings are caged in an open-topped, glass, 50-gallon-aquarium (90 cm long × 45 cm wide × 45 cm deep) or larger container in a laboratory at room temperature. The substrate is

1 We thank José P. O. Rosado and George W. Foley for technical assistance and Richard G. Zweifel for specimens, ideas and support.
2 C. J. Cole, in: Intersexuality in the Animal Kingdom, p. 340. Ed. R. Reinboth. Springer-Verlag, Berlin 1975.
3 R. G. Zweifel, Am. Mus. Novitates *2235*, 1 (1965).
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5 T. P. Maslin, J. exp. Zool. *166*, 137 (1967).
6 O. Cuellar and A. G. Kluge, J. Genet. *61*, 14 (1972).
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10 K. Freyse and G. Müller, Zool. Garten *26* (new ser.), 348 (1962).
11 T. P. Maslin, Copeia *1971*, 156 (1971).

beach sand with a depth gradient from 3 cm in the hottest quarter of the cage to 10 cm elsewhere. Heat is provided 9 h daily (except weekends) from a 250-W, infra-red heat lamp suspended about 35 cm above the sand at one end of the cage; thus the lizards can orient themselves within a thermal gradient of about 25–70 °C

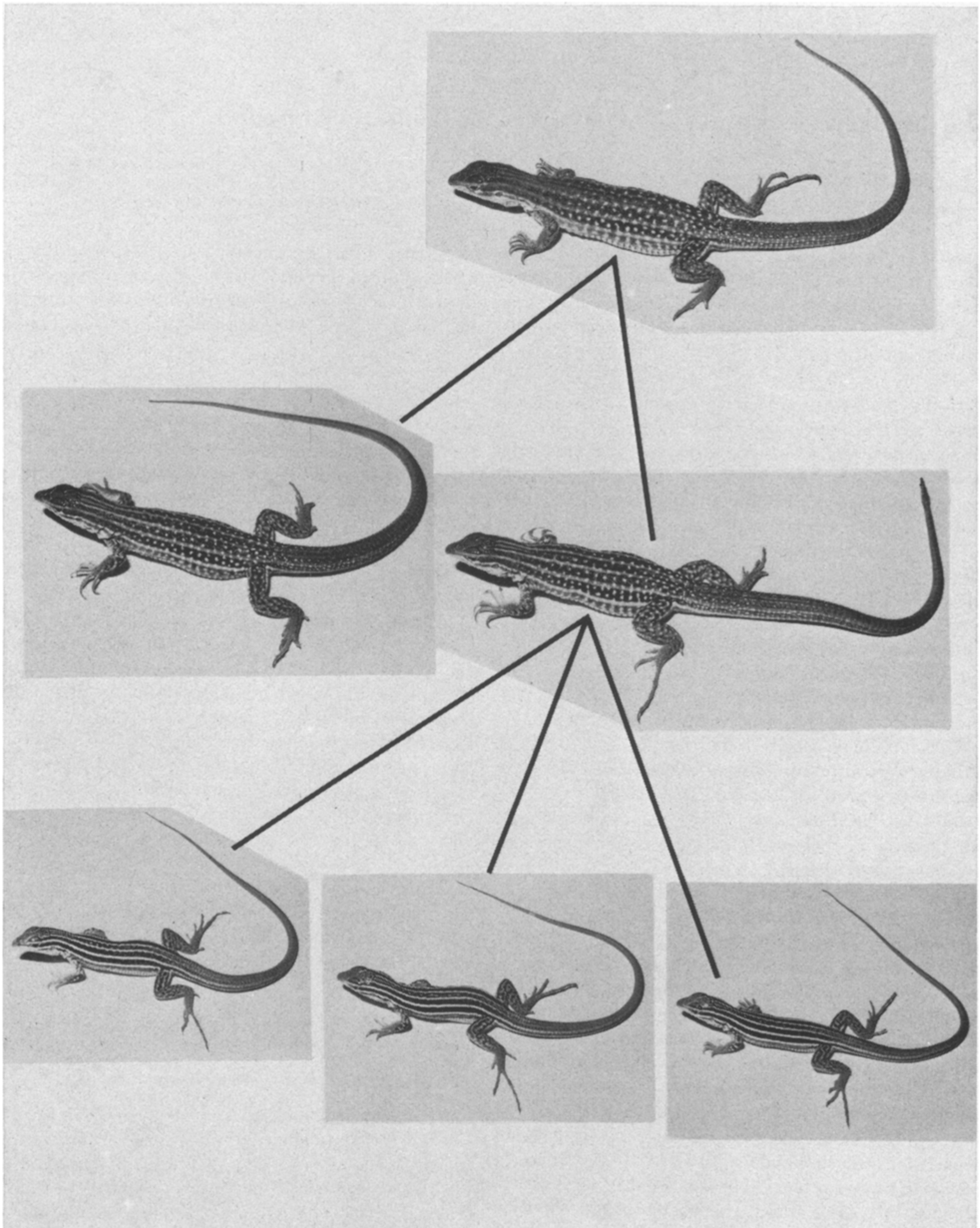

Fig. 1. Parthenogenetic lizards, Cnemidophorus exsanguis. Lines connect mothers and daughters representing 3 generations, all photographed within a month of each other; one-half actual size.

on the surface. Vita-Lites (fluorescent bulbs manufactured by Duro-Lite Lamps, Inc., 17–10 Willow St., Fair Lawn, New Jersey 07410), whose wavelengths closely approximate those of natural sunlight, including the ultra-violet, are suspended 50–60 cm above the substrate; two 74-W-bulbs span the width of 5 cages. Natural light and other laboratory lighting is not excluded from the cages, most of which are next to unshaded, east-facing windows (usually closed). 2 or more halves of cardboard egg cartons or similar sized pieces of bark, slate, or other objects provide cover.

Cleaning. Water dishes are scrubbed once a week, aquaria walls and shelter items as needed (weeks or months). The sand is sifted biweekly and changed biannually.

Food and water. Food consists primarily of half-inch, live crickets, Acheta domestica, purchased on mail order from a bait dealer and soft, recently molted pupae of the beetle, Tenebrio molitor, whose larvae (mealworms) and soft, recently molted adults are used occasionally. Assuming that food variety is beneficial, we also provide termites, pupae of Tenebrio obscurus, yellow-jacket (Vespula) larvae, Drosophila, caterpillars, moths and spiders, when available. Once a week the food items are dusted with a vitamin-mineral supplement by shaking them together in a plastic bag immediately before feeding. We use Paltone powder (prepared by Pitman-Moore, Inc., Washington Crossing, New Jersey 08560, for dogs and cats) and crushed limestone mixed in equal volumes, as suggested by Peter Brazaitis, Department of Herpetology, New York Zoological Society. Tap water is available constantly in low-sided saucers. A moisture gradient is maintained in the substrate, which is damp around the water dish but dry beneath the heat lamp.

Daily routine. The lizards require no care on weekends, but often we ascertain whether gravid ones oviposited. Lights and heat lamps are on each weekday from 8.00 a.m. to 5.00 p.m. Unless the ambient temperature is below about 20 °C, the animals emerge from shelter as soon as the room becomes light. Otherwise they usually appear soon after the lamps go on. A period of basking precedes drinking, defecating, and foraging, during which we feed them to satiation, sometimes including two feedings in a morning. Around noon, lizards retire under cover or into tunnels they excavated in the sand, usually not to re-emerge until the next morning. This routine is maintained year-round, without hibernation, although activities are reduced during periods of low temperatures and short daylengths. Also, gravid lizards tend to bask more than others.

Recognition. Each animal is individually marked by clipping off entire digits in a unique combination. 2 toes are removed from each lizard (not from the same foot) to minimize confusing animals if additional losses of digits occur later, usually in group feeding frenzies. Individual records are kept on hatching, geneology, growth, oviposition and disposition.

Oviposition. Field-collected adults may oviposit shortly after capture; laboratory-raised animals usually require eight months to a year. Even gravid lizards are fed to satiation; food consumption increases in early pregnancy and decreases immediately prior to oviposition. Individual bulges for many oviducal eggs become recognizable as the time for oviposition approaches. At this time, the substrate moisture and depth gradients are carefully maintained and the shelter material is kept over the area of deeper, damp sand. As lizards prefer to burrow under objects they are thus encouraged to oviposit in an appropriate damp site (eggs laid in dry sand desiccate very quickly and die). Oviposition occurs usually at night, but temperatures below about 22 °C may inhibit it; this can be counteracted by having a heat lamp on all night. After ovipositing, females usually have distinctly concave sides posteriorly and may appear emaciated; they drink and feed actively, however, and rebound quickly, often to produce another clutch of eggs about a month later.

Incubation of eggs. Buried eggs are carefully located, removed from the cage by hand, and placed on a bed of damp (but thoroughly wrung out) sphagnum moss within an inflated, transparent plastic bag, which is closed with a rubber band and kept in the laboratory at room temperature[12]. In healthy eggs, a germinal disk is visible through the bag and egg shell within a few days; later it becomes pink. During incubation, which takes 62–84 days depending on temperature, eggs need no attention other than moistening of the moss if it becomes too dry. Dead, moldy eggs need not be removed from bags with healthy eggs. Incubating eggs should be disturbed as little as possible, as there is a period during which the shells may rupture with even the most careful handling or merely opening the bag (accompanied by abrupt drop in humidity and presumably also temperature, through evaporative cooling). Eggs darken late in development as embryos develop pigmentation. Sometimes a drop of fluid seeps from a small hole in the egg shell several days before hatching; usually this is inconsequential.

Care of hatchlings. Babies are transferred to cages similar to those of adults immediately after hatching. Their care is similar to that of adults also. Food need not be provided in the first few days of life, because hatchlings have a reserve of yolk. It is very important for the next 45 days to ascertain that each hatchling is active, feeding, and drinking each day; their health can decline rapidly if it begins to slip. Babies that have difficulty finding the water dish are immersed in it and soon learn its location; it is one into and out of

12 R. G. Zweifel, Copeia *1961*, 112 (1961).

Reproduction of Cnemidophorus exsanguis hatched and then raised in the laboratory for a year or more

Individual	Life-span (dates)	Age (days) at laying first eggs	No. egg clutches	Total eggs	Natural egg deaths[a]	Other egg deaths[b]	Eggs now incubating	Offspring produced
AMNH 113359[c] (F_1 from AMNH 109468[c])	30.4.1973–16.2.1976 (died)	364	7	35	7	6	6	16 (F_2)
AMNH 113356[c] (F_1 from AMNH 113352[c])	30.4.1973–19.2.1976 (sacrificed)	379	7	34+ ?[d]	4	14	–	16 (F_2)
RR-4, LR-3[f] (F_2 from AMNH 113359)	16.8.1974–present	254	3	12	2	5	–	5 (F_3)
RR-4, LR-4[f] (F_2 from AMNH 113359)	16.8.1974–present	267	2	3+ ?[e]	1	–	–	2 (F_3)
RR-4, LR-5[f] (F_2 from AMNH 113359)	17.8.1974–present	261	2	7	–	–	4	3 (F_3)
Totals			21	91+ ?	14	25	10	42

[a]Embryos failed to develop, developed abnormally, or reached term and then died. [b]Mostly eggs deposited in poor places (e.g., dry sand) in the cages; a few were killed inadvertently or for experiments. [c]Individual reptile specimen, American Museum of Natural History. [d]One clutch, deposited on the surface of the substrate, contained half as many eggs as expected (3 instead of 6); perhaps some were eaten. [e]The first clutch had 3 eggs; the entire second clutch was eaten in the cage before we found and counted the eggs. [f]Individual toe-clips for recognition.

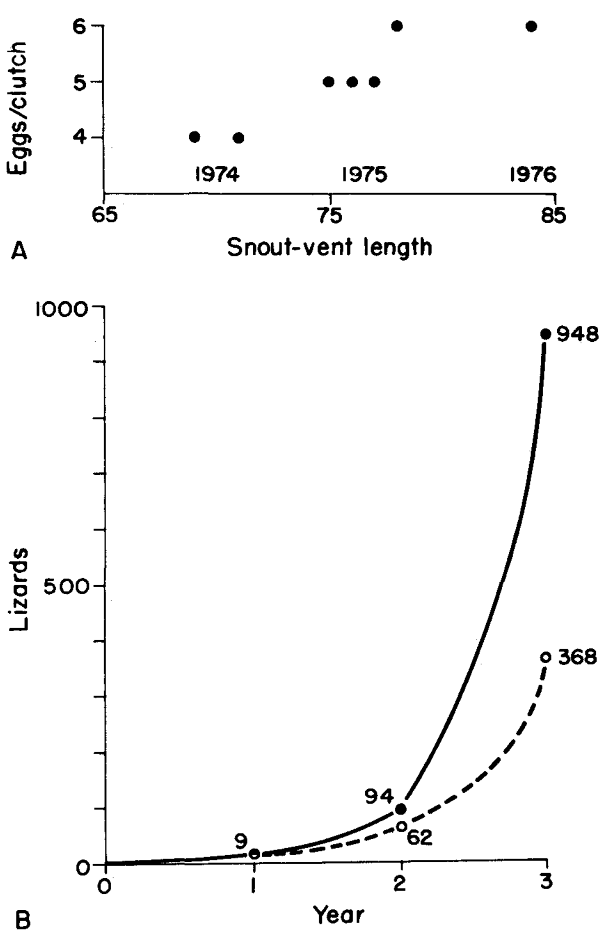

Fig. 2. *A* Egg production throughout the life of a parthenogenetic female C. exsanguis: AMNH 113359 (table). Body length is in mm. *B* Comparison of reproductive potential of a unisexual (all-female) species, ●, and a bisexual species, o, assuming 100% hatching, 100% survival, and each female having the same productivity as the C. exsanguis in figure 2A. The event at year 0 is the hatching of one female.

which they can climb easily. Cagemates are selected to be similar in size, so small lizards are not easily outcompeted or injured in feeding frenzies. The softest and smallest food items (termites; baby crickets; tiny, soft mealworms) are selected for hatchlings, as some have choked to death on hard, chitonous fragments of large pupae. Normal hatchlings often sleep in unusual positions and appear uncoordinated upon awakening; they behave similarly to adults, however, once they are active. Toenails are clipped about twice a year.

Special considerations. Numerous past attempts to raise parthenogenetic lizards resulted in the death of all hatchlings within 6 months of life. The one variable we changed that produced success was providing Vita-Lites, with ultra-violet wavelengths. In addition, survival among hatchlings is greatly increased by providing careful attention during their first 45 days of life. The substrate moisture and depth gradients also require close attention when lizards are gravid, to provide suitable sites for oviposition; at other times it is not so important, but we do maintain the gradients to some extent. However, if the sand is too damp and the animals sleep in it for prolonged periods (as in autumn, when often they are inactive for several days), lizards may contract a fatal respiratory infection. We cured the infection in some lizards by replacing the damp sand with dry sand and leaving a heat lamp on continuously for several days. Finally, our experiences have been primarily with C. exsanguis, but also we are keeping C. sexlineatus, C. sonorae, C. inornatus, C. neomexicanus, C. tesselatus, C. tigris and C. velox. Interspecific and intraspecific differences occur in productivity and behavior, which affect how individuals may be caged together and require minor procedural adjustments on occasion. Offspring raised together from hatching usually can be kept together well, or even combined with others, whereas field-captured lizards are less tame and less tolerant of cagemates, at least for a while.

Starting with adult females (P_1) of Cnemidophorus exsanguis captured in the field in 1972 (specimens are specified in the table), we have raised to maturity offspring of the F_1, F_2, and F_3 generations (figure 1), all developed from eggs laid by females maintained in complete isolation from males of any species of reptile. The colonies continue to thrive. We summarize the reproductive data for each female that hatched, reached maturity, and produced eggs that also hatched (table). After one of the 2 F_1 animals died, perhaps of old age (nearly 3 years in captivity without hibernation), we sacrificed the second to determine her karyotype (triploid, as characteristic of this species). One of these specimens provides the basic data for estimating productivity in colonies of captive C. exsanguis, in which there is a positive correlation between body length and the number of eggs in a clutch (figure 2 A).

Given the observed egg production of a single female (figure 2 A) and assuming 100% hatching of normal offspring, 100% survival, and each female having the same productivity, one can appreciate the tremendous difference in reproductive potential between unisexual (all-female) and bisexual (gonochoristic) species (figure 2 B). Not all eggs and offspring are viable or equally productive, however, and one must accommodate for this in planning to establish laboratory colonies.

Our observations indicate that at least 75.0% of the eggs deposited properly by the lizards can hatch into apparently normal offspring (table; a total of 91 eggs, less 25 other egg deaths, less 10 eggs now incubating = 56 eggs incubated, of which 42 hatched; actual viability is higher if some of our losses resulted from less than ideal incubation techniques). Approximately 71.4% of the hatchlings survive to adulthood (of the 42 hatchlings, table, 24 are alive and maturing now, 12 died and 6 were sacrificed for experiments; of the 12 deaths, 11 [91.7%] occurred at an age of less than 45 days and the other at less than 3 months; there has been 100% survival beyond 3 months of age; the 6 that were sacrificed were vigorous, healthy animals one of which was preserved at an age of 2 months, one at about 2.5 months, and 4 at ages from 3 to 9 months; thus, we conclude that 30 out of 42 hatchlings survived, or 71.4%). Applying these viability figures to the data used and projected earlier (figure 2), we estimate that a single lizard of a parthenogenetic species hatching at year 0 actually could be expected to result in a total population of 207 lizards at year 3. Reaching this productivity requires solving the problem of preventing the lizards from occasionally depositing eggs at dry or overheated sites in the cages, which may be accomplished by maintaining the temperature above 22°C at night, increasing depth of the sand and modifying the moisture gradient.

In the past, whiptail lizards (Cnemidophorus) have not been attractive as experimental animals primarily because they were difficult to acquire, many of their natural populations are not sufficiently large to withstand heavy sampling, and they have been impossible to breed and maintain reliably in captivity. Using the methods described here, laboratories can now establish colonies of parthenogenetic, and possibly bisexual species of whiptail lizards that reproduce in sufficiently large numbers to provide reptiles for a wide variety of research programs.