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Genetic Structure of Lowland Leopard Frog (*Rana yavapaiensis*) Populations in Southern Arizona

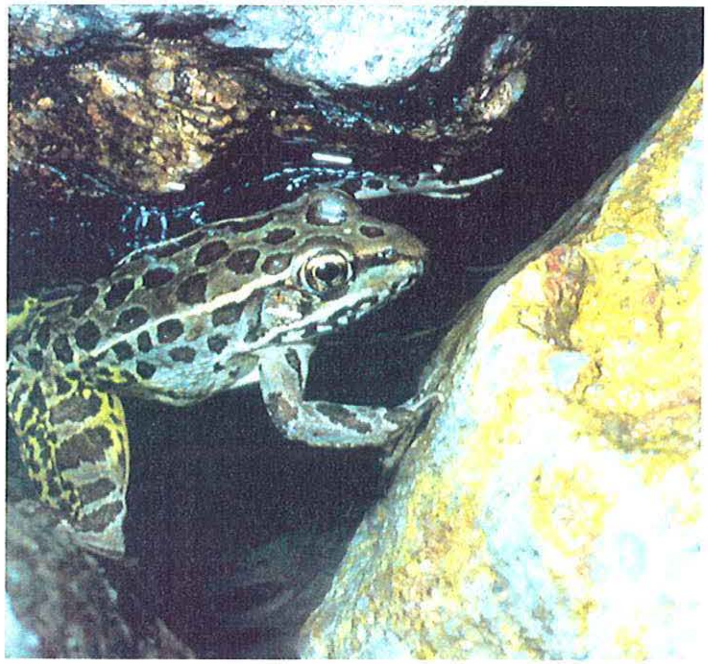

Photo by Cecil R. Schwable

Lay Report
Western National Parks Association and Saguaro National Park
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Background

This Western National Parks Association research project explored the conservation genetics of the lowland leopard frog *(Rana yavapaiensis)*, a rare and declining species that occurs in Saguaro National Park. Conservation genetics is a new scientific field that uses genetic techniques, such as DNA fingerprinting, to address many important conservation questions. Genetics can provide information, for example, on whether a small animal population has experienced in-breeding or past near-extinction events, or how isolated populations may be connected to each other across a landscape.

Lowland leopard frogs are ideal subjects for genetic studies, because detecting the presence and movements of this elusive species can be very difficult. Yet the connectivity of small and apparently seemingly isolated populations of frogs is believed to be critical to their survival. In addition, the size of these populations raises concerns that frogs, like the Florida Panther and other species that exist in small populations, may be vulnerable to genetically-linked health issues and other problems.

The lowland leopard frog is a true frog that requires standing water to survive. Paradoxically, Saguaro National Park is a desert park with no perennial streams. Yet the species is highly adapted to desert environments and is able to persist in canyon areas where small rock pools, called *tinajas*, keep small amounts of water throughout the year. Historically, lowland leopard frogs occupied riverine valley areas in southern Arizona, such as the Santa Cruz River in downtown Tucson. The loss of wet riparian areas due to human development has probably been the major factor leading to the decline of this species, which has now disappeared from many areas of Arizona and other western states. Another issue has been introduced predators such as bullfrogs and crayfish, which eat leopard frogs and are known to have eliminated entire populations. Major floods following large wildfires, which fill tinajas with sand and destroy stream habitat, have also caused populations in the Rincon and Santa Catalina Mountains to disappear in recent years.

Finally, lowland leopard frogs have been affected by a fungal disease called chytrids (known to scientists as *Batrachochytrium dendrobatidis*, or simply “Bd”). This chytrid fungus has only been recently identified, but is known to have been associated with large die-offs of frogs world-wide, as well as in Arizona. Recent evidence suggests that this fungus may have been recently (in the past few decades) introduced to North America, which raises concerns that our local frogs have not developed resistance to it.

The major goals of our study were to use genetics to look at how leopard frog populations in and near Saguaro National Park were related to each other and to look for “bottlenecks” as signs that populations used to be larger. A third purpose was to use genetic evidence to evaluate whether frog movements, which are very difficult to observe, follow drainages or straight lines across the landscape. The study built upon foundations laid by several other studies. Biologists and volunteers from Saguaro National Park have been monitoring leopard frog populations since 1996, and in 2002 the park and the University of Arizona began a research project on frogs and their habitat.

All of these projects provide information that is essential for preserving Saguaro National Park's populations of this rare species. In addition, previous research to develop genetic markers for leopard frogs, based on data from the park, was essential for the success of this study.

Results

We sampled tissue from 145 individuals in 9 populations, separated by distances ranging from ½ mile to more than 15 miles, in and near the Rincon Mountain District of Saguaro National Park. Samples were taken from live adults, adult frogs found dead, and tadpoles. Only a tiny amount of material is needed for DNA analysis. DNA from frogs and tadpoles was obtained by snipping a small piece at the end of the tail or a toe (something that is also commonly done by predators). The sampling was done in the field, and the frogs or tadpoles then immediately released.

In the lab, a tiny amount of DNA was extracted from tissue using a combination of physical and chemical techniques. This DNA was amplified, or cloned, into larger amounts that could be more easily worked with. Further analysis allowed researchers to study patterns in DNA from the cell nucleus. We worked with microsatellite DNA. Microsatellites, more famously used in crime scene analysis, are sequences of bases along DNA strands that evolve fairly rapidly and allow for detection of fine-scale differences among individuals. This technique allowed us to look at how closely individual frogs from different pools or canyons might be related.

A major, and surprising, result of our study was that lowland leopard frog populations in Saguaro National Park and vicinity are extremely isolated from each other. Genetic differences between frogs in different canyons were high, indicating that migration among canyons, and even between adjoining populations, does not occur very often. This was in contrast to what is known about other frog populations in North America. Interestingly, it contrasts with the results of a recent study of tortoises in the park, where individuals from the Tucson Mountain and Rincon Mountain Districts are genetically indistinguishable.

The results also contrast with what had been suspected in Saguaro National Park, that migration among canyons was an important way that small populations were replenished following local extinctions. The low connectivity we found indicates that the park's small populations are not repeatedly going extinct and being recolonized from nearby areas. Rather, it suggests that a few adults are probably persisting in canyons during drought periods and other times when they may not be observed. This low connectivity also makes frog populations vulnerable to random factors, such as drought or disease outbreaks, that may lead to extinction. It also strongly suggests that populations that "blink out" may never be naturally restored.

Four of seven populations tested showed evidence of a population bottleneck. Bottlenecks occur when a population or species experiences an event that leads to loss of all but a few individuals. Our results suggest that canyon populations may be showing signs of bottlenecks from the early 1970's, when most remaining Santa Cruz valley populations disappeared. With a generation time of approximately two years, bottlenecking may therefore have persisted through 17 generations. At least one population near the park where frog deaths associated with chytrids are common (Cienega Creek) appears to be at particular risk for inbreeding depression. Chytrids are present in the park, at least in the large population in Chimenea Creek, but have not led to high levels of inbreeding.

Isolation-by-distance analyses suggest that frog populations are primarily connected by movement through drainages and not along straight lines. This is not surprising considering how difficult it would be for an aquatic frog to survive far from water in the Sonoran Desert, but is in contrast to the straight-line movements of true frogs elsewhere. The evidence that lowland leopard frogs travel along stream channels emphasizes the importance of maintaining habitat in the drainages that connect remaining populations, many of which are currently in a degraded state at lower elevations.

We recommend that management efforts at Saguaro National Park focus on preserving even the smallest populations of leopard frogs whenever possible. Proposals to reintroduce frogs into areas where they have previously disappeared are worth pursuing. However, we recommend strongly that sampling be conducted over a series of years to verify that the native frogs are no longer present. To minimize diluting a gene pool that may be locally adapted, we recommend that translocations use the nearest population as a source, with geographic distance measured along drainages. While diluting a locally adapted gene pool is a concern, the risk of extinction due to demographic effects in these small, isolated populations is likely to be much higher than that due to loss of potential local adaptation.

Lowland leopard frogs in Saguaro National Park show signs that they used to be part of larger populations. Restoration of lower elevation areas to provide better connectivity for existing populations would make this species less vulnerable to unexpected events and provide it a more secure future in the Park.

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