# Appendix H: Stephens' Kangaroo Rat Genetic Monitoring Proposal
# DRAFT
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## *Genetic Monitoring*

A genetic monitoring program for Stephens' kangaroo rat (*Dipodomys stephensi*; SKR) could track genetic changes within and between populations over time. The goal would be to monitor population genetic parameters, including, at a minimum, genetic diversity, relatedness, inbreeding, structure, and effective population size. Current genomic techniques could also provide more accurate information on inbreeding and genetic load, and potentially also response to selective pressures associated with climate change. Genetic monitoring offers information about population health and vulnerability that cannot be inferred through monitoring occupancy or abundance. This approach can also provide early detection of population declines that may assist developing management plans to consider thresholds regarding population size and genetic diversity and the appropriate interventions. Our current understanding is that, throughout their range, SKR have generally high heterozygosity and allelic richness and low inbreeding coefficients (Shier & Navarro 2016), with evidence of little to no loss of haplotype diversity between sampling periods 1995-2013 (Metcalf et al. 2001; Shier & Navarro 2016). However, effective population sizes in some regions are quite low (Navarro et al. 2020) and continued urbanization throughout the species' range is likely to cause increasing population fragmentation. It is therefore critical to plan for genetic management even as we begin to systematically monitor the species.

### *Frequency*

Regular genetic monitoring should be conducted in tandem with range-wide population monitoring. Annual genetic monitoring could be useful to detect changes within a population over a short timescale, particularly if an area is trapped regularly to monitor population response to habitat manipulation or seasonal change.

### *Geographic Scale and Sampling Intensity*

There is evidence of genetic structure across the range of SKR, with 15 distinct clusters (Shier & Navarro 2016). Range-wide monitoring should collect samples from all clusters to capture the genetic diversity across the species' range. Sampling intensity should be proportional to population size, with more samples collected from larger populations, particularly those that occupy wide spatial areas, different habitat types or fragmented habitat, and are more likely to show genetic structure across space. We therefore suggest using survey grids stratified by habitat suitability as a way to standardize genetic sampling and match sampling effort to size of suitable habitat. We recommend collecting genetic samples from all individuals sampled,

particularly if we utilize genomic techniques and can collect hair rather than tissue samples from all individuals (see Table 1).

*Sample Banking*

We propose to use current state-of-the-art genomic techniques to extract the maximum amount of data from each sample. We acknowledge, however, that genetic techniques will change and more powerful techniques may become available. We recommend banking up to 50% of samples so there is genetic material from locations and time points available for future analyses. Prior to processing samples, collection locations of all available samples should be assessed and banked samples should represent a wide geographic range and spatial heterogeneity.

*Methodology*

There are multiple options of specific genetic techniques (Table 1) based on the funding available to develop the monitoring program and maintain it over time. Any of these methods would provide data on genetic diversity, structure, and effective population size. Currently, 24 highly polymorphic microsatellites have been identified for SKR and have been used to assess the range-wide genetics of the species (Shier & Navarro 2016; highlighted in green on Table 1). Continuing to use these microsatellite markers to monitor SKR genetics requires no additional development time or cost, however the data that can be collected is limited by the number of markers (n=24), tissue samples (ear snips) are required for monitoring, and lab processing is labor intensive and fairly costly ($100/sample).

Switching to newer, more powerful genomic techniques such as SNP arrays (Allendorf et al. 2010; Carroll et al. 2018; highlighted in yellow in Table 1) or sequence capture (Ali et al. 2016; Meek & Larson 2019; highlighted in orange in Table 1) could provide more nuanced information on inbreeding and genetic load, and potentially also allow monitoring of adaptive markers as populations respond to selective pressures associated with climate change. Either of these methods would first require the one-time investment in marker development (highlighted in grey in Table 1) to identify regions of the genome to monitor. Marker development will require additional tissue sampling throughout the range, about 6 months to process samples, and costs about $5,000-10,000. Once markers are developed, DNA from hair could be used instead of tissue for regular monitoring, the cost per sample is relatively low ($10-100/sample, with lower per-sample costs when they are processed in bigger batches), and many more markers (n=100-20,000) provide greater power in detecting changes between populations or over time. Protocols for collecting genetic samples are included in the Appendix.

**Table 1**. Alternative genetic (green) and genomic (yellow and orange) techniques with pros and cons of each technique highlighted. Genetic technique (green) is currently in use and requires no additional start up time or investment. Genomic techniques (yellow and orange) require the initial step of developing markers, and two alternative options for marker development are shown in grey. Once markers are developed, genomic techniques would be less expensive than genetic techniques and could utilize hair or feces, rather than tissue samples (ear snips), for analysis.

| | Traditional markers | SNP arrays | Sequence capture via baits | Marker Development-Anonymous DNA sequencing | Marker Development-Reference genome |
|---|---|---|---|---|---|
| Summary | PCR amplification of nuclear and mitochondrial loci | Hybridizing array with fluorescent probes, genotyping by real-time qPCR | Use of capture baits to target and sequence loci identified through reduced-representation approaches | High-throughput sequencing of reduced representation genomic DNA fragments | Sequencing of whole genome |
| Examples | Microsatellites, Cyt-b and D-loop | Fluidigm dynamic arrays; Illumina Golden Gate; Applied Biosystems OpenArray; Ampliflour | Rapture | RADseq | Next-generation technologies |
| **General Considerations** | | | | | |
| Development considerations | N/A – Microsatellites libraries already developed | Marker development (Need high-quality DNA samples to identify markers) | Marker development (Need high-quality DNA samples to identify markers) | One time effort to develop markers for monitoring | One time effort to develop markers for monitoring |
| Cost | $100 per sample | $10-100 per sample (cost decreases with increasing number of samples) | $50-100 per sample (cost decreases with increasing number of samples) | One time cost of $10,000 to develop markers for all future genomic analyses | One time cost of $5,000-10,000 to develop markers for all future genomic analyses |
| Number of markers | 24 | 96 (most platforms)-1,000 | 100-20,000 | 1,000-20,000 | Complete genome |

| **Pros** | | | | | |
|---|---|---|---|---|---|
| | Consistent with legacy data sets<br><br>All start-up is complete; could begin monitoring and analysis immediately<br><br>Standardized protocol at fixed set of markers- suited for long term monitoring<br><br>Processing and analysis can be completed by people with less expertise<br><br>Low amount of DNA needed per sample | More markers provide greater power in detecting changes between populations or over time<br><br>Standardized protocol at fixed set of markers- suited for long term monitoring<br><br>After initial set-up, costs per sample are comparable or less than microsatellites<br><br>Arrays can process low quality/quantity DNA; could re-run remnant legacy samples with minimal DNA<br><br>Could switch to hair or fecal samples rather than ear snips<br><br>May allow processing of museum specimens with low quality DNA<br><br>Better estimates of heterozygosity and Ne than microsatellites<br><br>Less time consuming than microsatellites<br><br>Candidate adaptive alleles can be targeted | Similar to SNP arrays, but more markers<br><br>More genomic data gives more accurate information on demographic changes and inbreeding<br><br>Can target specific regions of interest or functional variation e.g. exons, immune genes | Does not require a reference genome for marker discovery | Does not require re-sampling all populations<br><br>Potential to identify “non-neutral” markers to monitor loci under selection |

| **Cons** | | | | | |
|---|---|---|---|---|---|
| | Fewer markers mean limited ability to detect pedigree/kin, identify inbreeding/genetic load, less fine-scale<br><br>Microsatellite screening is time consuming and requires multiple replicates to validate allele cells<br><br>Continue to rely on ear snips for tissue samples | Not consistent with legacy data sets- would need to re-process remnant legacy samples (but this would be feasible)<br><br>High initial cost in money and time to develop markers<br><br>High quality samples necessary for initial marker development | Similar to SNP arrays | Need tissue sample from individuals across the range, containing as much variation as possible<br><br>Mostly neutral markers identified | Time and expertise for bioinformatics<br><br>Reference genome comes from a single individual- not able to search for variable loci |

***References***

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**Field Sample Collection Protocols for DNA Work**
**San Diego Zoo Institute for Conservation Research**

## Sampling tissue for DNA:

1. Use permanent pen to label tape on tissue tube with the following information:
   - Species
   - Location- site, grid/line, trap number
   - Date
   - Sex
   - Unique ID (assign a number associated with any field notes)
2. **Collect tissue:**
   - Prior to sampling each individual, sterilize the scissors in 70-100% ethanol (keep a separate vial for this purpose).
   - Holding the scissors on a tangent from the edge of the pinna, snip a sliver (~0.5mm) off the edge.
   - Place tissue into tube with field buffer (see Notes below) so that tissue piece is completely immersed. The snip should be visible in the vial after collection but should not draw blood.
   - Once the sample is collected, close the lid tightly to make sure the ETOH does not leak out.
   - Samples can be stored at room temperature if they will be transferred to SDZICR within a few days; otherwise they should be refrigerated. Try to keep the samples upright to prevent leaking.
   - Take a GPS coordinate at each trap location where a genetic sample is collected. Match it to the unique ID or the grid and trap number.
   - Transfer samples and GPS coordinates to Debra Shier, Ph.D. at San Diego Zoo Institute for Conservation Research for banking and future analysis (dshier@sandiegozoo.org).

Notes: Field buffer and sampling supplies (provided upon request)

- 100% ethanol (ETOH)
- 1.5 ml plastic vials
- Osung SCTC115 Tissue Scissors
- Fisher Scientific colored label tape (wrap around vial)

## Sampling hair for DNA:

1. Use permanent pen to label envelope with the following information:
   - Species
   - Location- site, grid/line, trap number
   - Date
   - Sex
   - Unique ID (assign a number associated with any field notes)
2. Collect hair:
   - Change gloves before sampling each individual.
   - Hair should be kept as dry as possible. Carefully pluck approximately five - ten or more hairs with follicles attached from individual, and immediately place into envelope.

Envelope acts as a filter and allows hair sample to dry. DNA is present only in hair follicles, so avoid including shed hair or hairs that are without visible follicles.

- Use tape to seal each envelope. Do not lick!
- Samples should be refrigerated or frozen if possible, but can be stored at room temperature if necessary.
- Take a GPS coordinate at each trap location where a genetic sample is collected. Match it to the unique ID or the grid and trap number.
- Transfer samples and GPS coordinates to Debra Shier, Ph.D. at San Diego Zoo Institute for Conservation Research for banking and future analysis (dshier@sandiegozoo.org).