Development of a Genotyping Protocol for Mojave Desert Tortoise Scat

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ABSTRACT. – Noninvasive fecal genotyping can be a useful tool for population monitoring of elusive species. We tested extraction protocols on scat samples from the threatened Mojave Desert tortoise, Gopherus agassizii, to evaluate whether scat-based mark–recapture and population genetic monitoring studies are feasible. We extracted DNA from G. agassizii scat samples collected in California and Nevada using several extraction protocols and evaluated the reliability of resulting genotypes using quality scores, maximum likelihood reliability estimates, and paired scat and blood genotypes from the same individuals. Finally, we assessed probabilities of identity and sibship, and locus amplification quality, and calculated genotyping error rates for 19 microsatellite loci to determine the best set of loci to use with G. agassizii scat extractions. We found that genotype quality depended more on the sample quality than on the extraction method, and that the Qiagen DNeasy Plant Mini extraction kit is an efficient method for extracting tortoise DNA from tortoise scat. We identified 6 G. agassizii microsatellite loci that can be used to generate a unique molecular tag for individual tortoises. We characterized the reliability of an additional 13 microsatellite loci for use in population genetic analyses where additional power at the expense of some increase in error may be advantageous. As proof of concept, with very low error rates, we matched 3 opportunistically collected scat samples to blood genotypes from animals captured during population surveys within the study area and discovered at least 3 new individuals, even after 2 yrs of extensive survey work. These results suggest that genotyping of field-collected scat can complement existing methods used in long-term demographic and movement studies of G. agassizii and other, closely related, tortoise species.

KEY WORDS. – California; Gopherus agassizii; genetics; microsatellites; Nevada; noninvasive sampling

The Mojave Desert tortoise, Gopherus agassizii, occurs in the Mojave Desert and in parts of the Sonoran Desert of the United States (US Fish and Wildlife Service [USFWS] 2011; Edwards et al. 2015). Because of decreases in population densities, the Mojave population of G. agassizii was listed as threatened under the federal Endangered Species Act in 1990 and also receives state protection in California, Nevada, Arizona, and Utah (USFWS 1990). Numerous factors threaten G. agassizii including loss, degradation, and fragmentation of habitat due to an expanding human footprint throughout its range (USFWS 2011). Currently, populations are monitored using traditional methods such as plot surveys, radiotelemetry, and genetic samples (i.e., blood) collected when animals are handled. However, these elusive herbivores can be difficult to detect because of their cryptic appearance, behavior, and propensity to use burrows. This results in many animals being missed during surveys (Freilich et al. 2000; Anderson et al. 2001; Nussear and Tracy 2007; Nussear et al. 2008). Therefore, to complement current methods implemented for studying G. agassizii populations, we explored the potential of using scat samples, which can be opportunistically collected during other survey efforts, to yield genetic data for monitoring.

Noninvasive scat sampling and individual identification through genotyping have become useful tools for population monitoring of wildlife species (Schwartz et al. 2007). Scat-based genotyping can provide information on individual movement, home range, relatedness, abundance, food habits, parasite load, and sex ratios (Waits and Paetkau 2005; Schwartz et al. 2007; Beja-Pereira et al. 2009; Luikart et al. 2010). Using scat-based genetic sampling without the need to handle individual animals is appealing for studies of elusive, rare, or endangered species (Piggott et al. 2008; Giambattista and Gentile
animals were collected opportunistically from the ground during the same sampling period.

Blood samples were stored at room temperature on Whatman cards. Scat samples were collected either in closed, 50-mL tubes with desiccating beads or in individual, non–air-tight plastic boxes or paper lunch bags and allowed to dry outdoors overnight (samples in boxes were dried with lids open). Increased air contact appeared to inhibit fungal development. A similar collection method has worked well in studies genotyping mule deer scat (Bohonak and Mitelberg 2014; Mitelberg and Vandergast 2016). To simulate the approximate age and quality of samples that would be collected in the field, a subset of 3 fresh samples were additionally exposed to 7–9 d of full sun. Scat samples were stored dry in the laboratory at room temperature and extracted within 3 wks of collection.

**Blood Extractions.** — All blood extractions were performed with the Qiagen DNeasy Blood and Tissue extraction kit, according to the manufacturer-provided protocol, with these minor modifications to improve yields: following addition of Buffer AL, samples were incubated at 70°C for 10 min; elution volume was halved to 100 μL, and the elution step was performed twice, for a total of 200 μL final elution volume.

**Scat Extractions.** — We experimented with 6 scat extraction protocols consisting of combinations of different pre-extraction surface washes, extraction kits, and postextraction clean-up kits to determine if any of these improved quality (Table 1). Five of these extraction protocols used the Qiagen QIAamp Fast DNA Stool Mini extraction kit (hereafter stool kit) on surface-washed cells, and one protocol used the Qiagen DNeasy Plant Mini extraction kit (hereafter plant kit) on whole, homogenized scat. Scats were split in half prior to extraction to increase sample sizes across extraction protocols and to provide paired samples for protocol comparisons. To minimize opportunities for contamination, we used new disposable gloves for each scat sample and performed all extraction steps (with the exception of centrifugation) under a PCR hood.

**Stool Kit with Surface Wash and Clean-Up Steps.** — Scat surface washes utilized Inhibitex buffer (included in the stool kit) by either 1) placing the scat segment into a 50-mL centrifuge tube with buffer and agitating on a nutating rocker for 10–15 min (“tube surface wash”; Table 1), or 2) placing the scat in a weighing dish with buffer and leaving the dish on the nutating rocker for 10–15 min (“dish surface wash”; Table 1). Following washes, remaining buffer and epithelial cells were transferred by pipette to 2-mL centrifuge tubes and extracted with the stool kit following the manufacturer’s protocol. As an additional measure for removing PCR inhibitors from extracted DNA, an aliquot of each extraction was processed through either the OneStep PCR Inhibitor Removal kit (Zymo) or the Genomic DNA Clean & Concentrator-10 kit (Zymo). We followed
manufacturer's protocols for the OneStep kit and made the
following modifications to the Clean & Concentrator-10 kit: a 1-min spin step was added following the use of
Wash Buffer (Zymo) to facilitate removal of all ethanol,
and two sequential elution steps with 10-min incubation
periods were performed using 20 μl warmed (60°C–70°C)
Elution Buffer (Zymo).

**Plant Kit Extractions.** — The DNeasy Plant Mini kit (plant kit) includes steps to shear cell walls and reduce PCR
inhibitors often present in plant tissues, which could be useful
given the herbivorous diet of the desert tortoise. We cut off a
small piece (< 300 mg) of each scat sample (making sure to
include material from the surface and interior of the scat) and
extracted the piece using the manufacturer’s protocol. For a

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**Table 1.** Extraction protocols including pre-extraction surface wash method, extraction kit, and clean-up kit (if used), number of Gopherus agassizii scat samples extracted, approximate time, cost, and number of steps per protocol.

<table>
<thead>
<tr>
<th>Extraction protocol</th>
<th>Pre-extraction surface wash</th>
<th>Qiagen extraction kit</th>
<th>Zymo clean-up kit</th>
<th>No. of samples</th>
<th>Approx. time per sample (min)</th>
<th>Extraction protocol cost/sample (kits and reagents only) (US dollars)</th>
<th>No. of steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tube</td>
<td>Stool</td>
<td>None</td>
<td>4</td>
<td>45</td>
<td>5.38</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>Dish</td>
<td>Stool</td>
<td>None</td>
<td>6</td>
<td>45</td>
<td>5.38</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>Dish</td>
<td>Stool</td>
<td>OneStep</td>
<td>3</td>
<td>50</td>
<td>7.38</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>Tube</td>
<td>Stool</td>
<td>Clean &amp; Concentrator</td>
<td>10</td>
<td>55</td>
<td>6.88</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>Dish</td>
<td>Stool</td>
<td>Clean &amp; Concentrator</td>
<td>11</td>
<td>55</td>
<td>6.88</td>
<td>21</td>
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<tr>
<td>6</td>
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<td>Plant</td>
<td>None</td>
<td>16</td>
<td>40</td>
<td>4.58</td>
<td>13</td>
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</table>

* 8 scats were split and paired between protocols 4 and 6.
* 8 scats were split and paired between protocols 5 and 6.
paired comparison of the plant and stool kit protocols, the remainder of each of these scat samples was extracted with stool kit protocols 4 or 5 (Table 1).

Amplifications. — Blood and scat DNA extractions were amplified at either 6 or 19 microsatellite loci available for G. agassizii (Edwards et al. 2003; Schwartz et al. 2003; Hagerty et al. 2008). We assessed each locus individually in single-locus, 10-μl PCR reactions containing 5 μl 2× Multiplex PCR Plus cocktail (Qiagen), 1 μl 10 μM primer, 2.5 μl water, and 1.5 μl DNA (blood extractions were standardized to 4 ng/μl). Thermocycler conditions were as follows: enzyme activation for 5 min at 95°C followed by 30 and 40 cycles for blood and scat, respectively, of 30 sec at 95°C, and 3 min at 56°C, 45 sec at 72°C, with a final elongation at 68°C for 30 min. One microliter of PCR product was aliquoted into 10.5 μl Hi-Di™ formamide (Thermofisher) with 0.5 μl GeneScan™ 500 LIZ® size standard (Thermofisher) and was submitted for genotyping to Eton Bioscience (San Diego, CA). GeneMarker v.1.90 was used to score chromatographs. Negative controls were included with each round of PCR to monitor contamination and all PCR reactions were prepared under a PCR hood.

Deriving Consensus Genotypes. — To determine whether a reliable molecular tag could be obtained from G. agassizii scat, we amplified all extractions at a subset of 6 microsatellite loci yielding the shortest PCR products (referred to as screening loci from this point on). Loci with shorter amplions generally have higher amplification and lower error rates (Frantz et al. 1998; Broquet et al. 2006). Additionally, successful genotyping of at least 5 of these 6 loci satisfies the recommended minimum threshold for probability of identity (P<sub>SIB</sub> ≤ 0.01; Waits et al. 2001). Each scat extraction was genotyped 3 times at each locus, with extractions from the same scat sample before and after postextraction clean-up being treated as separate extractions. Because each scat sample was split in half and subjected to 2 different extraction treatments, this yielded a total of 6 replicate PCRs for each scat sample.

To arrive at an “extraction consensus genotype”, we used the following guidelines of Frantz et al. (2003): 1) for heterozygotes, each allele must be present at least twice among replicate PCR reactions; and 2) for homozygotes, the allele must be present at least 3 independent times and all 3 times as a homozygote (to eliminate the possibility of ADO). In addition to a consensus genotype for each extraction, we were able to determine a “sample consensus genotype” for each scat sample using all 6 replicate amplifications. To arrive at the sample consensus genotype, we used the same rules as for the extraction consensus genotype.

Assessing Genotype Quality. — We used three approaches to estimate genotype quality across extractions, samples, and loci. First, we calculated genotype quality (Q-score) following Miquel et al. (2006). At each locus, genotypes were compared with the consensus genotype for that sample and a score of 1 was assigned for that replicate in the case of a match; all other genotypes (ADO, FA, PCR failure [FAIL], and lack of consensus genotype [UNK]) were assigned a score of 0. Q-scores were calculated for each extraction and sample, at each locus, by averaging scores for replicate genotypes. Second, we used the maximum likelihood software RELIOTYPE (Miller et al. 2002), which uses repeated amplifications to determine the probability that samples met certain reliability thresholds. We used default settings (95% reliability, 200 bootstrap replicates) and allele frequencies estimated from a larger sample of 159 tortoises collected throughout the Ivanpah Valley in 2016–2017 and genotyped from blood (Dutcher et al., in press). We applied the reliability criteria to the entire data set, limiting the probability of false acceptances to less than 5% with 95% probability, and retained all instances of FA, as the focus of this study was to document potential issues with genotyping because of poor DNA quality. Samples accepted without further PCR replicates were deemed as suitable quality samples (SQS). Finally, for a subset of samples for which we had paired scat and blood samples available, we directly compared sample consensus genotypes of scat samples to their respective blood genotypes.

Evaluation of Scat Extraction Methods. — To explore extraction protocols, we compared Q-scores, cost, time, and steps involved in each extraction protocol. We used box plots to visualize Q-scores for the 6 extraction protocols we evaluated. We used a paired Wilcoxon signed-rank test to determine if there was a significant difference in Q-scores between samples extracted with the stool and plant kits. We limited this comparison to 16 samples that were divided and extracted with both kits (protocols 4, 5, and 6; Table 1).

Locus Suitability for Genotyping G. agassizii Scat. — To explore additional loci that may be useful for genotyping G. agassizii scat samples, we further genotyped 8 scat and 3 blood extractions at 19 loci, with 5 PCR replicates per locus. For scat, we limited this experiment to samples that were extracted with the plant kit (protocol 6; Table 1) and were deemed SQS samples when assessed at the 6 screening loci as described above. We calculated Q-scores and analyzed reliability with RELIOTYPE. We calculated error rates for all 19 loci, following Broquet and Petit (2004), based on consensus or blood genotypes when available (in the case of 3 of 8 scat samples). We visualized average Q-scores for loci using box plots. To assess our power to reliably identify individual tortoises from scat samples, probabilities of identity (P<sub>ID</sub>) and probabilities of sibship (P<sub>SIB</sub>) were calculated for these 19 microsatellite loci using allele frequencies estimated from the larger (159 sample) data set (Dutcher et al., in press).

Identifying Opportunistically Collected Scat. — To further test the utility of scat-derived genotypes, we conducted an identity analysis in CERVUS (v. 3.0.7; Kalinowski et al. 2007). We tested whether any of the opportunistically collected scat samples with SQS genotypes matched any tortoises genotyped from blood.
samples in Ivanpah Valley to date (309 individuals; Dutcher et al., in press). For this analysis, we used allele frequencies from this larger data set, allowing up to 1 mismatching locus to avoid excluding matches due to genotyping error.

**RESULTS**

Quality of *G. agassizii* Scat Sample Genotypes at Screening Loci. — Overall, we evaluated 11 blood samples and 25 scat samples (in 50 extractions). Of 25 total scat samples, RELIOTYPE identified 11 (44%) as SQS samples (Table 2). Nine of 11 SQS samples qualified with all 6 loci genotyped. Two samples (2017S_01 and 2017F_09A) qualified with a 5-locus genotype. For these samples, locus GOAG4 was not evaluated by RELIOTYPE due to the presence of aberrant (previously unrecorded) alleles in at least one of the replicates. Of the 16 samples which were either field collected (with unknown identity) or for which field collection was simulated by exposure to field conditions (Table 2; samples identified with “E” in sample name), 5 (31%) were deemed SQS samples. Of the 9 fresh samples collected directly from animals being handled (and without exposure to field conditions), 6 (67%) were found to be SQS samples. SQS samples had average Q-scores that ranged from 0.64 to 1.

All SQS samples with average Q-scores ≥ 0.6 matched their blood genotypes at all loci, suggesting that samples that meet these quality criteria should have accurate genotypes (Table 2). In 3 samples that failed to meet the SQS and Q-score criteria above, we found 6 mismatches between the sample consensus genotype and the blood genotypes (Table 3). Five of these errors were identified as false alleles and one was a case of large allelic dropout.

<table>
<thead>
<tr>
<th>Scat sample</th>
<th>Tortoise ID</th>
<th>Scat sample Q-score</th>
<th>Scat sample reliability</th>
<th>SQS</th>
<th>Percent loci with consensus genotype</th>
<th>Percent loci with matching blood genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017F_13</td>
<td>CN019</td>
<td>1.00</td>
<td>1.000</td>
<td>Yes</td>
<td>100</td>
<td>100</td>
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<tr>
<td>2017S_03</td>
<td>CN720</td>
<td>0.94</td>
<td>1.000</td>
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<td>100</td>
<td>100</td>
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<tr>
<td>2017F_03B</td>
<td>BS589</td>
<td>0.92</td>
<td>1.000</td>
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<td>100</td>
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<tr>
<td>2017F_03A</td>
<td>BS589</td>
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<td>1.000</td>
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<td>100</td>
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<tr>
<td>2016S_01</td>
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<td>100</td>
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<td>2017S_01</td>
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<td>100</td>
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<td>2017S_06E</td>
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<td>0.892</td>
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<td>83</td>
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<tr>
<td>2017S_02</td>
<td>CN716</td>
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<td>0.994</td>
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<td>83</td>
<td>17</td>
</tr>
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<td>17</td>
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<td>0.97</td>
<td>1.000</td>
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<td>2017F_01</td>
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<td>0.997</td>
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<td>100</td>
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</tbody>
</table>

Table 3. Mismatches between *Gopherus agassizii* scat and blood-derived genotypes (presented as allele sizes) and inferred error type. LADO = large allele dropout; FA = false allele.

<table>
<thead>
<tr>
<th>Scat sample</th>
<th>Blood sample</th>
<th>Locus</th>
<th>Scat genotype</th>
<th>Blood genotype</th>
<th>Q-score</th>
<th>Error type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017S_06E</td>
<td>CN748</td>
<td>GOA8</td>
<td>163/163</td>
<td>163/171</td>
<td>0.50</td>
<td>LADO</td>
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<tr>
<td>2017S_02</td>
<td>CN716</td>
<td>GOA2</td>
<td>207/210</td>
<td>210/213</td>
<td>0.83</td>
<td>FA</td>
</tr>
<tr>
<td>2017S_02</td>
<td>CN716</td>
<td>GOA12</td>
<td>148/152</td>
<td>111/152</td>
<td>0.17</td>
<td>FA</td>
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<td>2017S_02</td>
<td>CN716</td>
<td>GOAG4</td>
<td>168/180</td>
<td>168/170</td>
<td>0.67</td>
<td>FA</td>
</tr>
<tr>
<td>2017S_02</td>
<td>CN716</td>
<td>GP30</td>
<td>207/211</td>
<td>207/207</td>
<td>0.33</td>
<td>FA</td>
</tr>
<tr>
<td>2016S_03</td>
<td>CN812</td>
<td>GOA12</td>
<td>142/158</td>
<td>111/142</td>
<td>0.17</td>
<td>FA</td>
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</table>
dropout (where the larger of two fails to amplify in a heterozygous individual).

**Evaluation of Scat Extraction Methods.** — Thirty-four scat extractions were performed using the stool kit (with 5 variations) and 16 with the plant kit. All extraction methods had overlapping distributions in average Q-scores, with protocol 1 performing slightly worse than the other protocols (Fig. 2). In addition, we found no significant difference in Q-scores between paired stool kit and plant kit extractions (W_{15} = 58.5; p ≤ 0.3815). The cost and time investment for all stool kit protocols is higher than that of the plant kit protocol. The plant kit has fewer pipetting steps (limiting potential for contamination or operator error), consumes just a small portion of the scat sample (so multiple extractions could be performed to increase DNA yield), and extractions may also be used in the future for *G. agassizii* diet analyses.

**Locus Suitability for Genotyping *G. agassizii* Scat Extractions.** — For blood extractions genotyped multiple times, we encountered no PCR failures or erroneous genotypes at any of 19 loci (i.e., Q-score = 1.0). Of 8 scat extractions, each with 5 replicate PCR reactions, RELIOTYPE accepted the multilocus genotype of 4 extractions without additional PCR replicates (Supplemental Table S1; supplemental material is available at https://doi.org/10.2744/CCB-1394.1.s1). One of these samples qualified with a 14-locus genotype, 2 samples qualified with 18-locus genotypes, and 1 sample qualified with a 19-locus genotype. Error rates ranged from 0% to 47.4% for ADO and 0% to 5.9% for FA. Average Q-scores ranged from 0.23 for Locus GP81 to 1.0 for Locus GOA1 (Fig. 3; Supplemental Table S1). The 3 loci with the lowest average Q-scores (< 0.54), also had either exceptionally low successful allele call rates or low genotype accuracy rates. The cumulative probabilities of identity (P_{IDcum} and P_{SIDcum}) for the remaining 16 loci were 5.8 × 10^{-22} and 9.7 × 10^{-8}, respectively.

**Identifying Opportunistically Collected Scat.** — Three of the 7 unknown scat samples that met SQS standards were matched unambiguously at all 6 screening loci to previously captured tortoises. All three scats were found within 400 m of where the matching animal was captured. Additionally, we found 2 sets of samples with fuzzy matches (mismatched at 1 locus). One fuzzy match occurred between 2 blood samples taken approximately 8 km apart in different plots in the Ivanpah Valley data set, and so likely indicates these 2 individuals are close relatives. The other fuzzy match occurred between a blood and scat sample located within the same sampling site within 500 m. The 3 remaining high quality unknown scat samples did not match any individuals in our blood samples and likely represented previously unsampled individuals.

**DISCUSSION**

In this pilot study, we evaluated the feasibility of using *G. agassizii* scat to complement current methods...
used to monitor this species. First, we found that a reliable molecular tag can be obtained from DNA extracted from *G. agassizii* scat. Notably, scat samples that met SQS criteria and had average Q-scores ≥ 0.6 also exactly matched blood samples from the same individuals, suggesting that these 2 quality criteria are appropriate for retaining accurate tortoise genotypes from scat. Second, of the 6 extraction protocols evaluated in the study, we detected no differences in extraction quality, but we found the Qiagen DNeasy Plant Mini kit to be the most cost- and time-efficient method. Finally, we found that 16 of 19 loci tested could be genotyped from scat with relatively high confidence for population genetic analyses while limiting genotyping error.

**Reliability of *G. agassizii* Scat Sample Genotypes.**—As many as 7 amplifications per locus have been recommended to obtain reliable genotypes in noninvasive studies (Taberlet et al. 1999). Statistical approaches such as those employed by RELIOTYPE are designed to reduce replication number, without compromising accuracy, by taking into account data already available (i.e., allele frequencies and data from a limited number of initial replicates), inferring the reliability of the consensus genotype derived from those data and suggesting an appropriate number of additional replicates to arrive at a predetermined confidence level for the multilocus genotype. In our study, we found that the default settings of RELIOTYPE were stringent enough to accept without further replicates only those scat genotypes that matched their respective blood genotypes. We also found that approximately 31% and 67% of scat samples collected from the ground and directly from animals, respectively, yielded DNA of sufficient quality to obtain reliable genotypes. A multilocus genotype of at least 5 loci will yield a molecular tag with $P_{SIB}$ below the recommended threshold of 0.01 (Waits et al. 2001); therefore, these multilocus genotypes can be used as molecular tags for purposes of mark–recapture studies or for population size estimates. As proof of concept, we were able to match several unknown scat samples to genotyped animals in the same plots with low $P_{ID}$ and $P_{SIB}$.

**Evaluation of Extraction Methods.**—We observed high variability in genotype quality among samples regardless of extraction protocols. Sample quality is likely the most critical factor in determining whether a reliable genotype can be obtained from *G. agassizii* scat. Edwards et al. (submitted) evaluated a different scat extraction protocol on *G. agassizii* and *Gopherus morafkai* scat; they used epithelial cells swabbed from the surface of scats and reported similar success rates to our study (avg. 35% across field collected tortoise scats). Although we detected no difference in sample quality related to extraction protocol, the plant extraction
protocol has advantages in terms of cost, time, and yield efficiencies. Pearson et al. (2015) found that the Qiagen DNeasy Plant Mini kit performed well for individual genotyping from field-collected lizard scat when alternative extraction protocols optimized on captive lizard scat failed on wild samples. The authors attributed the failure to the diet of wild lizards, which was richer in vegetation than that of captive lizards. In our study, we did not find a significant difference between genotype quality for the subset of samples that were extracted with both the stool and plant kits. This may be because all stool kit extractions were performed on the surface washes, avoiding most of the plant material inside the scat (unlike alternative protocols in Pearson et al. 2015). We also note that, while pelleted herbivore scat lends itself well to surface washes (Flagstad et al. 1999; Wedrowicz et al. 2013), this was not the case with tortoise scat because 1) it is not pelleted, 2) size is variable, and 3) tortoise scat segments are larger and less compacted. At times, scat segments partially crumbled during the wash and absorbed significant amounts of buffer during the wash procedure (up to 4 ml of Inhibitex Buffer per sample). Overall, the plant kit protocol was less expensive and less labor-intensive, took less time, and had fewer steps (potentially reducing contamination risk). Finally, plant kit extractions could also be used to amplify and analyze the tortoise’s herbivorous diet, the quality of which has been shown in controlled experiments to be directly linked tortoise health (Drake et al. 2016).

**Locus Suitability for Genotyping G. agassizii Scat Extractions.** — There is a trade-off in the amount and accuracy of information that can be obtained from noninvasively collected samples (Waits and Leberg 2000). For purposes of molecular tagging for estimates of census population size using capture–recapture methods, if the number of loci is too low some individuals will have the same molecular tag, resulting in an underestimate of population size. Conversely, introducing more loci increases the probability for error, which in turn creates false individuals resulting in population size overestimation. Thus, highly polymorphic loci with low genotyping error rates are recommended for studies using molecular tags to obtain estimates of population size (Waits and Leberg 2000). We found the initial 6 screening loci to be a reliable set of loci for molecular tagging, yielding a molecular tag with low probabilities of misidentifying 2 random individuals (P_{ID}) or 2 siblings (P_{SIB}) as the same individual.

In contrast to estimating census population size and paternity, genotyping error at low frequencies may not be as problematic in the estimation of population genetic diversity, differentiation, and effective population size, and the inclusion of more loci can increase precision in these estimates. In simulations, Smith and Wang (2014) found that, with genotyping error rates below 0.2, reasonable estimates of genetic variation and population subdivision could be obtained. Based on our tests of 19 loci for *G. agassizii*, we found that 16 loci could be genotyped with relatively high confidence.

**Conclusions.** — While sample sizes in this pilot study are small, the results are encouraging enough to warrant pursuing scat genotyping as a viable source of data for *G. agassizii* population monitoring. In particular, detection of 3 “recaptures” of previously genotyped animals indicates that scat genotyping could be used for recapture in regions with a good set of reference DNA samples. Finally, the developed scat extraction and genotyping protocol can be extended across the species range and possibly to other tortoise species for which these microsatellite loci cross-amplify. This reliable, scat-based genotyping method can complement ongoing efforts characterizing desert tortoise populations throughout the Mojave Desert.

**Acknowledgments**

We thank K. Drake, F. Chen, B. Gottsacker, and Ironwood Consulting for sample collection efforts. Research was supported by the US Bureau of Land Management (BLM), the USFWS, the National Fish and Wildlife Foundation (NFWF), and the US Geological Survey (USGS), Ecosystems Mission Area Energy and Wildlife Program. We are grateful to A. Fesnock (BLM) and M. Slaughter (BLM) for their support of our desert tortoise research program. All tortoises were handled in accordance with USGS, Western Ecological Research Center, and UC Davis IACUC, USFWS Permit TE030659-11, Nevada Department of Wildlife Scientific Collection Permit 317351, and a Memorandum of Understanding with the California Department of Fish and Wildlife held by T.C.E. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government. Data are available as a USGS Data Release (https://doi.org/10.5066/P9SNWMJY).

**Literature Cited**


Received: 22 May 2019
Revised and Accepted: 25 September 2019
Published Online: 25 November 2019
Handling Editor: Jeffrey E. Lovich