

Waste Not, Want Not: Toe-clips as a Source of DNA

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The rapid decline in population size observed in several amphibian populations has received much attention over the last decade (Baringa, 1990; Blaustein and Wake, 1990; Wake, 1991). Whether these crashes are natural, cyclic phenomena, or the result of anthropogenic factors is controversial (Pechmann and Wilbur, 1994). Molecular genetic data can help address these questions (Moritz, 1994). Here we present a method of collecting DNA from toe-clips, a typically discarded tissue. Toe-clipping is a semi-permanent technique, commonly used to individually mark small vertebrates during field studies. This technique is widely accepted among herpetologists since it does not appear to have any long term effects on behavior and poses minimal risk to the individual (Ferner, 1979; ASIH et al., 1987). Here we demonstrate that toe-clips can be used as a reliable source of DNA without sacrificing the individual.

Recent advances in molecular technology, specifically the polymerase chain reaction (PCR; Mullis et al., 1986), have allowed small amounts of DNA to be used for genetic analysis (e.g., Wayne and Jenks, 1991; Wiesner et al., 1991; Taberlet and Bouvet, 1992; Wil-

son et al., 1995). In light of these recent advances, we saved toe-clips taken during a population study of the Puerto Rican frog, *Eleutherodactylus coqui*. By saving the toe-clips, we had the opportunity to both permanently mark individuals and collect population genetic data using this typically discarded tissue.

Eleutherodactylus coqui is endemic to Puerto Rico (Rivero, 1978). Toe-clips from 12 individuals, and one whole individual (control tissues) were collected from L. L. Woolbright's long-term study population at a National Science Foundation Long Term Ecological Research site in the Luquillo Experimental Forest of northeastern Puerto Rico. All toes were clipped using sharp scissors. The blades of the scissors were scrubbed, with a sterile cotton swab soaked in 95% ethanol, after each use. A new cotton swab was used for each scissor cleaning. This procedure was necessary to help reduce cross contamination of DNA between individuals and infections to the individuals being clipped. Toe-clips were stored in 70% ethanol in 1.5-ml screw top cryovials for up to 40 d in the field (≈ 25 C) with no apparent "adverse" effects. Upon return to the laboratory, toe-clips were stored at -80 C until DNA extraction.

DNA was prepared according to the basic mammalian tissue protocol in Ausubel et al. (1989). Just prior to digestion with proteinase K, toe-clips were transferred to tared 1.5 ml centrifuge tubes and weighed. The digestion buffer used was 10 mM Tris, 100 mM EDTA, 20 mM NaCl, 1% N-Lauryl sarcosine, and proteinase K was added to 0.2 mg/ml. Each sample contained four toe-clips from each individual and 0.5 ml of digestion buffer with proteinase K was used for each digestion. Digestion was performed for 18-24 hours at 55 C. Protein was extracted twice using 0.5ml of 25 Phenol: 24 Chloroform: 1 IsoAmyl Alcohol. Percipitation of DNA was performed by adding 125 μ l of 7.5 M Na Acetate and adding 95% ETOH for a total volume of 1.5 ml. The tube was inverted to mix and spun to form a pellet. The ethanol was decanted and the pellet washed twice with cold 70% ETOH. The pellet was air dried and brought back into solution with 200 μ l of TE. As a control, to determine if the DNA obtained from toe-clips was actually *E. coqui* and that all tissues gave the same sequence (Quinn, 1992), we sacrificed one adult frog and extracted DNA from several different tissues (heart and lung, liver, skeletal muscle, ovaries and toe-clips) in the same manner as the toe-clips. DNA was quantified and purity estimated spectrophotometrically (SLM Aminco® 3000 Array spectrophotometer).

A segment of the mitochondrial cytochrome *b* gene was amplified using conserved internal primers; L16762-5'-GGA-TTT-GAG-GCG-GAT-TTT-CTG-TAG-3' and H17080-5'-ATT-AGC-GAC-GGA-TCG-TAA-GAT-[CT]GC-GTA-[CGT]GC-3' designed for *E. coqui* (this study) and named according to the 3' nucleotide position in the complete *Xenopus laevis* mitochondrial genome (GenBank: Accession: X02890). All PCR reactions used negative (H_2O + primers) and positive (human DNA + primers) controls. Only the PCR products from the tissues and toe-clips of the same individual were directly sequenced (same primers as in amplification) using the Taq Dye Deoxy Cycle Sequencing Kit (Applied Biosystems) and analyzed on a 373A automated sequencer (ABI; DNA Sequencing Facility, SUNY-Albany). All tissue sequences (same

1 2 3 4 5 6 7 8 9 10

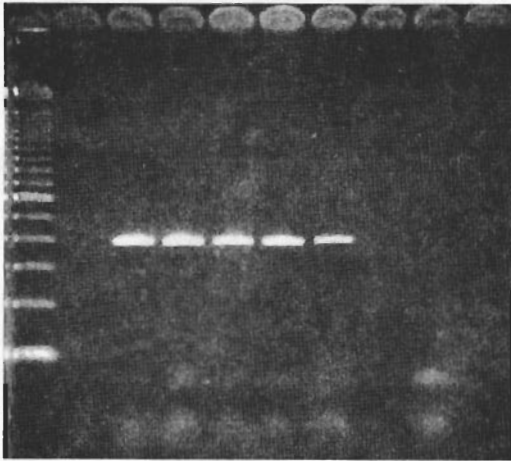


FIG. 1. A 3% agarose gel showing PCR amplification products from several tissues from the same individual and a toe-clip sample from another individual. Lane assignment as follows: 1 = 100 base pair ladder (GibcoBRL); 2 = blank; 3 = Skeletal muscle; 4 = Heart and Lung; 5 = Ovary; 6 = Toe-clip; 7 = BI toe-clip; 8 = blank; 9 = Water; 10 = blank.

individual) were compared using the software package SeqEd (Applied Biosystems) and aligned using GeneWorks (IntelliGenetics), (Default parameters). *Eleutherodactylus coqui* sequence was compared to the published *Xenopus laevis* sequence.

DNA yields for toe-clips ranged from 18.2–93.6 µg (AVG: 47.1 ± 22.6 µg from 0.0156 ± 0.0205 g of tissue; N = 13). All tissues and toe-clips gave the same ≈350 basepair amplification product after initial PCR amplification (Fig. 1). Once the conditions were optimized there has been a 100% success rate amplifying this fragment from toe-clips of 100 coquíes (Gonser, unpubl. data). Fragments from skeletal muscle, ovaries, heart and lung, and toe-clips were sequenced in both directions, and found to be identical to each

other for a 288 basepair section. This sequence was then aligned to bases 16,792 to 17,080 of the *Xenopus laevis* mitochondrial genome (cytochrome *b* gene) (Fig. 2). There are 85 nucleotide differences, predicting 26 amino acid changes between the two frog sequences.

We have demonstrated that total cellular DNA obtained from frog toe-clips does indeed provide suitable substrate for PCR amplification. Although this is not a novel finding in terms of extracting DNA from small amounts of tissue for sequence analysis, it illustrates a source for genetic data previously unexplored by herpetologists.

Large-scale declines in amphibian populations have received much attention over the last decade (Blaustein and Wake, 1990; Blaustein et al., 1994; Blaustein and Wake, 1995). Implications of these declines have concentrated on persistence, extinctions, and conservation. More long-term population studies of amphibians are needed that emphasize the importance of demographic information in determining the persistence of amphibian populations. For long-term population studies, suitable semi-permanent marking methods are needed. We suggest that the “old standard” of toe-clipping can serve two purposes. First, toe-clipping provides an adequate individual mark. Second, as we have demonstrated, toe-clips can provide important genetic materials without sacrificing the individual (most blood and tissue sampling methods are at least as harmful).

Most herpetologists typically discard toe-clips to the forest floor. We suggest that any such tissue commonly discarded during marking can be saved and will provide a rich source of population genetic data.

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<i>E. coqui</i>	ATT CAC TTC ATT CTC CCA TTT GTT ATT ATT GGA GCA ACC GCT CTC CAC CTG CTC TTC CTC
<i>X. laevis</i>	T... .. C.C ..T ..T ... A... .. GCCT ..G. AT.T ..T T.A ..T ...
<i>E. coqui</i>	CAC GAA ACA GGA TCT TCC AAC CCC ACA GGA CTT AAC TCT AAC CTA GAC AAA GTT CAA TTT
<i>X. laevis</i>TA A.AA ..T ... T.AA G... ..C. ..TA .CT ..C
<i>E. coqui</i>	CAC ACC TTC TTT TCC TAC AAA GAT ATT CTT GGA TTT GCC ATT CTC TTC ACC CTC CTA TCC
<i>X. laevis</i>	... C.A ..A. ..C ..TC C... T.A ..C ..C CTT ... A.A C.T ..A GCA ..T A.T
<i>E. coqui</i>	TTA GTT TCC ACA TTT TTC CCC AAT ATT CTA GGG GAC CCA GAT AAT TTT ACC CCC GCT AAC
<i>X. laevis</i>	C.C C.A G... ..T.C. ..A ..C C... T... ..ACAT
<i>E. coqui</i>	CCA TTA AGT ACT CCC CCC CAC ATT AAA CCA GAG TGA TAT TTT CTA TTC
<i>X. laevis</i>	..T C... ..TC ..C ..T ..A ..TAC ..C

FIG. 2. DNA sequence (consensus using several tissues from the same individual) of the *Eleutherodactylus coqui* cytochrome *b* gene compared to nucleotide numbers 16792 to 17080 of the *Xenopus laevis* mitochondrial genome (GenBank: Accession: X02890). Identities to the coqui sequences are indicated by dots.

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