A Method of DNA Sampling in Lizards with Tail Autotomy

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Studies on breeding success using multilocus DNA fingerprinting in lizards are rare and DNA ordinarily is obtained from blood samples (Olsson et al. 1994, 1996). However, blood sampling in lizards can be difficult, especially in small individuals, and may not provide sufficient high molecular weight DNA for fingerprinting. Blood has been obtained from the orbital sinus (Moore 1986), tail cuts (Tokarz 1987), a minute cut in the corner of the mouth (Olsson et al. 1994), or the caudal vein (Wilson and Wingfield 1994), but this manipulation could cause injury or haematoma and the amount of blood taken might affect some traits in study animals.

In our studies with *Psammodromus algirus*, we tried the above methods and found that the most efficient blood sampling was from the caudal vein. However, in subadult and juvenile individuals the amount of blood obtained is insufficient for some procedures. Here, we present an alternative method of DNA extraction from tail fragments, as autotomy (tail separation) may occur in nature and might not represent a significant handicap to the individual animals manipulated.

Five adult individuals of *P. algirus* were captured near Navacerrada (Madrid province, Spain). Tails from these individuals were removed and stored in absolute ethanol at 4°C until pro-

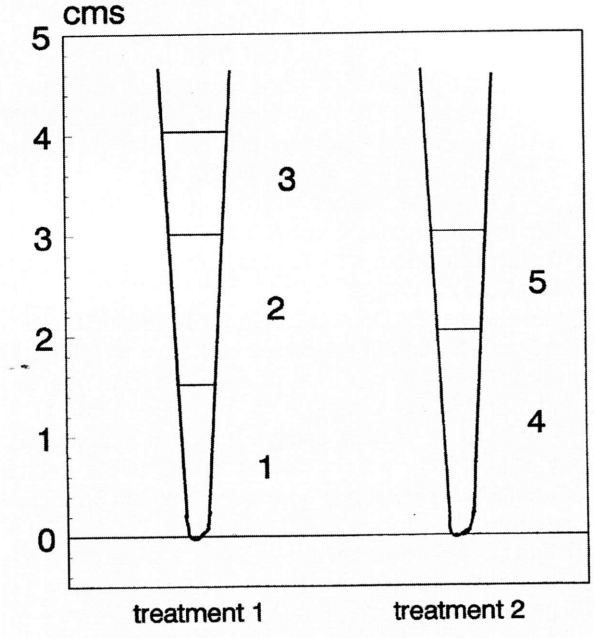

FIG. 1. Tail fragments analyzed. Treatment 1. Each individual tail was cut in three fragments: Fragment 1, from tip up to 1.5 cm; fragment 2, between 1.5 and 3 cm; fragment 3, between 3 and 4 cm. Treatment 2. Each individual tail was cut in two fragments: Fragment 4, from tip up to 2 cm; fragment 5, between 2 and 3 cm.

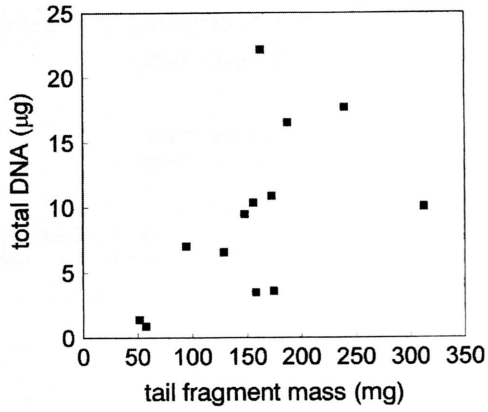

FIG. 2. Relationship between DNA content and tail fragment mass.

cessed. To assess the minimum size of tail fragment necessary to isolate adequate amounts of DNA for fingerprinting, tails were cut in two different ways. In treatment 1, we made cuts in three individuals (A–C) at 1.5, 3, and 4 cm from the tip of the tail. In treatment 2, we cut tails of two individuals (D, E) at 2 and 3 cm from tip of the tail (Fig. 1). A sample of 30 µl of blood, stored in absolute ethanol, was used as a standard for comparison. Tail fragments were dried on Whatman paper to remove the ethanol, weighed, and digested separately in 650 ml SET buffer (150 mM NaCl, 50mM Tris/HCl pH 8.0, 1mM EDTA Na_2 pH 8.0), 15 µl proteinase K (20 mg ml^{-1}) and 7.5 µl of 250 g l^{-1} SDS and incubated overnight at 55°C. DNA was purified by one extraction with phenol, followed by one with phenol/chloroform and one with chloroform iso-amyl alcohol (24:1). It was then precipitated with 0.1 volumes 3M sodium acetate and 2 volumes of cold (-20°C) absolute ethanol and pelleted at approximately 11,000 rpm for 5 min. The DNA was washed with 75% ethanol, dried at 37°C for 30 min and resuspended by overnight incubation in TE (1mM EDTA Na_2, 10mM Tris/HCl, pH 8.0). The amount of buffer added for resuspension ranged from 20–70 µl, depending on the size of the DNA pellet. 15 µl of the redissolved DNA was digested with 10U of Hae III in the presence of 4 mM spermidine, according to manufacturer instructions. The concentration and size of cut DNA was assessed by fluorometer and mini-gel. The mini-gel was prepared in 1x TAE (40mM Tris, 40 mM acetic acid, 1 mM EDTA Na_2) with .8% LE agarose, stained with 0.5µg.ml^{-1} Ethidium Bromide and electrophoresed for 30 min at 100V. Uncut and cut DNA from tail fragments together with Lambda DNA - Hind III markers were revealed by UV light.

High molecular weight DNA (>23 Kb.) was obtained from all samples analyzed. Distal tail fragments of 1.5 cm weighing 52–95 mg produced 0.89–7.03 µg DNA, whereas distal tail fragments of 2 cm and 129–156 mg yielded 6.58–10.37 µg (Table 1). Although 1.5 µg of DNA may be enough to obtain a minisatellite DNA (May et al. 1993), we considered 5.0 µg as the minimal amount of total DNA per sample necessary to obtain predictable results. Therefore, the shortest distal fragments (1.5 cm) did not yield sufficient DNA for minisatellite analysis. Longer distal fragments (2 cm) were more than enough to run one or two gels, 3 cm of tail (fragments 1+2 or 4+5) produced sufficient DNA for several assays, a necessity when the same individual must be scored in different family assemblages.

We plotted the amount of DNA produced versus fragment mass (r_s= 0.65, N = 13, p = 0.017) to obtain predictable estimates of fragment size required for adequate DNA collection. The lower limit mass to yield 5 µg of DNA corresponded about to 80 mg of tail fragment (Fig. 2). In our experiment, 2 cm of tail fragment produced more DNA than 30 µl of blood (Table 1), a volume which is available only from large individuals (unpubl. data).

Different genetic assays require different amounts of DNA. For example, PCR amplification for microsatellites or sequencing requires small quantities of template DNA, in the order of nanograms. As shown in Table 1, any of the fragments in our analysis produced more than enough genomic DNA for these amplifications.

TABLE 1. DNA yield obtained from tail fragments of five individuals (A–E) of *Psammodromus algirus* according to segment position (1 to 5, Fig. 1) and mass.

Individual	Tail fragment	Mass (mg)	Total DNA (µg)
A	1	58	0.89
A	2	158	3.50
A	3	175	3.60
B	1	52	1.40
B	2	148	9.50
B	3	173	10.90
C	1	95	7.03
C	2	240	17.70
C	3	313	10.06
D	4	129	6.58
D	5	163	22.17
E	4	156	10.37
E	5	188	16.53
BLOOD	—	30µl	7.28

Our sampling method can be used for species with autotomy in which bleeding may be difficult or traumatic. Although tail loss may reduce home range size and movement rates in *P. algirus* (Salvador et al. 1995), this effect was recorded only in males with a regenerated tail shorter than 150 mm (Salvador et al. 1996). As the length of intact tail in this species may reach 200 mm in adults, the consequences of removing fragments of 2–3 cm for DNA sampling may be negligible. The use of this sampling method in other species of lizards with caudal autotomy is recommended if the fragment of tail removed produces enough DNA and does not modify the behavior or any component of fitness of the species under study.

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Use of a Fluorescent Marking Technique on Small Terrestrial Anurans

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The choice of a marking technique for research should be based on the objective of the study, effectiveness of the technique, ease of application, and the potential risk to the organism. While toe-clipping is convenient and inexpensive, and thus remains a common technique for marking adult anurans (Donnelly et al. 1994), there is some evidence that it may affect behavior or survivorship (Clark 1972; Golay and Durrer 1994; but see Lemckert 1996). The use of a marking technique that potentially affects the organism's behavior or survival is usually unacceptable; thus, alternatives to toe-clipping should be considered. Marking amphibians with fluorescent pigments appears to be a promising alternative (Donnelly et al. 1994; Windmiller 1996). A pressurized application of fluorescent powder was applied successfully to aquatic and terrestrial salamanders (Ireland 1973, 1991; Nishikawa and Service 1988; Taylor and Deegan 1982). However, the effectiveness, details of application, and risks of this technique have not been quantified for other amphibians.

I recently used a pressurized fluorescent marking technique on small terrestrial leaf-litter frogs (*Eleutherodactylus podiciferus*; Leptodactylidae) in Costa Rica to evaluate its applicability. Inert fluorescent powder was applied to a hind leg of frogs using a pressurized source of air similar to that described in Nishikawa and Service (1988). However, Nishikawa and Service (1988) used a small 2-liter source of pressurized N_2, whereas I used a source of pressurized air delivered by a SCUBA tank. The nozzle of the spray gun was modified to accommodate a variety of interchangeable nozzle openings. The 7/64 inch (2.8 mm) opening was optimal, as it did not clog the nozzle yet produced a concentrated point. The yellow, fluorescent, granular powder (50–350 mm) produced a mark 3–4 mm in diameter on the hind leg of the frog when delivered at a pressure of 100 psi, approximately 0.5 cm from the skin.

I compared the use of fluorescent pigments in the field to toe-clipping with respect to i) ease of application and disturbance to frogs, ii) risk to frogs (i.e., survival), iii) longevity of the mark, and iv) logistics and cost. I caught and marked 68 adult *E. podiciferus* (10–24 mm SVL) in 1996, alternating between the fluorescent powder technique, and toe-clipping a single foot digit (digit IV). Clipped toes were disinfected with a dab of Bactine® to reduce the likelihood of infections (Martin and Hong 1991).