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TECHNICAL NOTE

Buccal swabbing as a source of DNA from squamate reptiles

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Abstract Buccal swabbing was compared with other tissues as a source of DNA for microsatellite genotyping from two squamate reptiles. For both species, the lizard *Lacerta agilis* and the snake *Coronella austriaca*, buccal swabbing proved more reliable than tissues including tail tips, toe clips and ventral scale clips.

Keywords Lizard · Snake · Microsatellites

Buccal swabbing has become a routine non-invasive technique for the DNA sampling of many species including amphibians (Pidancier et al. 2003; Broquet 2007), chelonian reptiles (Poschadel and Moller 2004) and the tuatara (Miller 2006). I investigated whether buccal swabbing provides a reliable source of DNA from sand lizards *Lacerta agilis* and smooth snakes *Coronella austriaca*, two rare reptiles in Britain (Beebee and Griffiths 2000), and compared it with relatively invasive methods.

Ten lizards and 10 snakes provided the samples. Toe-tips from a hind foot were taken using sharp scissors from five lizards, and 10 mm tail tips were gently broken off from the remaining five. Pieces of ventral scale (c. 4 mm^2) were cut from five snakes, and 10 mm tail tips were cut from the other five. All animals were also buccal-swabbed using BuccalAmp™ extraction kits (Cambio, Cambridge, UK), as prescribed by the manufacturer. Each animal was allowed to bite/chew on the swab for about 1 min, after which the swabs were briefly air-dried. All samples were returned immediately to the laboratory, and all the animals were released.

DNA was obtained from the various tissues using a standard proteinase K digestion and phenol/chloroform extraction procedure. Swabs were extracted according to the manufacturer's instructions. Approximately 100 ng of DNA from the tissue samples, and 4 µl of swab extract DNA, were used in PCRs of final volumes 20 µl or 40 µl respectively. For *L. agilis*, primers the eight microsatellite loci *La-1, La-2, La-3, La-4, La-5, La-8, La-9* and *La-10* were used (Gullberg et al. 1997); For *C. austriaca*, the eight microsatellite loci *Ca16, Ca19, Ca20, Ca26, Ca30, Ca40, Ca45* and *Ca47* were used (Bond et al. 2005). Genotypes were analysed as described elsewhere (Rowe et al. 1997). In the event of failure to obtain a genotype on the first attempt, a second attempt was always made.

Table 1 summarises the results. Lizard toe clips were significantly more successful than tail tips (Wilcoxon signed rank test, WSRT, two-tailed exact $P = 0.031$). There was no significant difference in the success rates of snake scale clips and tail tips (WSRT, $P = 0.688$). However, for both species buccal swabbing was by far the most successful method. For the lizard, buccal swabbing generated 68 genotypes (85% of the possible total of 80) compared with the tissue methods (combined) that yielded 41 genotypes (51% success), a highly significant difference (WSRT, $P = 0.008$). For the snake, buccal swabbing generated 73 genotypes (91% success) whereas the combined tissues generated 55 genotypes (69% success), again a highly significant difference (WSRT, $P = 0.016$).

In the lizard, there were no significant differences among the loci but highly significant differences (Kruskal–Wallis ANOVA, KWA, $P < 0.001$) among individuals. In the snake, there were differences among loci (KWA, $P = 0.043$) because one locus, *Ca16*, worked very poorly for tissue

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Table 1 Summary of genotyping results

Locus	*La-1*	*La-2*	*La-3*	*La-4*	*La-5*	*La-8*	*La-9*	*La-10*
L. agilis								
Tail tip (/5)	1	2	3	3	1	2	3	2
Toe clip(/5)	2	3	3	4	3	3	3	3
Total: tip + clip (/10)	3	5	6	7	4	5	6	5
Buccal swab (/10)	9	9	9	9	9	8	9	6
Locus	*Ca16*	*Ca19*	*Ca20*	*Ca26*	*Ca30*	*Ca40*	*Ca45*	*Ca47*
C. austriaca								
Tail tip (/5)	2	2	4	5	4	4	3	3
Scale clip(/5)	0	4	4	3	5	4	5	4
Total: tip + clip (/10)	2	6	8	8	9	8	8	7
Buccal swab (/10)	8	10	9	9	10	10	8	9

Numbers are of successful genotypes obtained after up to two attempts

samples. There were also differences among individuals (KWA, $P = 0.016$).

Observed heterozygosity averaged 0.561 in the lizard tissue samples and 0.588 in the lizard buccal extracts. For the snake, the values were 0.473 (tissue) and 0.507 (buccal). Genotypes agreed well between tissue and buccal DNA samples, with only four differences in the lizard and two in the snake.

Buccal swabbing therefore worked well as a technique for producing PCR-quality DNA in both the reptiles studied here. Tissue extracts obtained by more destructive methods were less satisfactory, perhaps because contaminants interfered with the PCR. Buccal swabbing was easy to do (all specimens of both species were easily induced to chew on the swabs), produced no obvious signs of stress or tissue damage, and yielded good results. It seems likely that buccal swabbing will prove a good method for obtaining DNA from many species of squamate reptiles.

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References

Beebee TJC, Griffiths RA (2000) Amphibians and reptiles. Harper-Collins, London

Bond JM, Porteous R, Hughes S, Mogg RJ, Gardner MG, Reading CJ (2005) Polymorphic microsatellite markers, isolated using a simple enrichment procedure, in the threatened smooth snake (*Coronella austriaca*). Mol Ecol Notes 5:42–44

Broquet T (2007) Buccal swabs allow efficient and reliable microsatellite genotyping in amphibians. Conserv Genet 8:2

Gullberg A, Tegelstrom H, Olsson M (1997) Microsatellites in the sand lizard (*Lacerta agilis*): description, variation, inheritance, and applicability. Biochem Genet 35:281–295

Miller HC (2006) Cloacal and buccal swabs are a reliable source of DNA for microsatellite genotyping of reptiles. Conserv Genet 7:1001–1003

Pidancier N, Miquel C, Miaud C (2003) Buccal swabs as a non-destructive tissue sampling method for DNA analysis in amphibians. Herpetol J 13:175–178

Poschadel JR, Moller D (2004) A versatile field method for tissue sampling on small reptiles and amphibians, applied to pond turtles, newts, frogs and toads. Conserv Genet 5:865–867

Rowe G, Beebee TJC, Burke T (1997) PCR primers for polymorphic microsatellite loci in the anuran amphibian *Bufo calamita*. Mol Ecol 6:401–402