NORTH-WESTERN JOURNAL OF ZOOLOGY 7 (2): 325-328
Article No.: 111208

www.herp-or.uv.ro/nwjz

Buccal swabs as a reliable non-invasive tissue sampling method for DNA analysis in the lacertid lizard *Podarcis muralis*

Ulrich SCHULTE*, Felix GEBHARD, Lisa HEINZ, Michael VEITH and Axel HOCHKIRCH

Department of Biogeography, Trier University, 54286 Trier, Germany
* Corresping author, U. Schulte, E-mail: schulte@uni-trier.de, Tel: 0049-651-201-4691, Fax 0049-651-201-3851

Received: 11. November 2010 / Accepted: 10. May 2011 / Available online: 14.July 2011

Abstract. We tested the performance of buccal swabs for microsatellite analysis in an introduced population of the Common Wall Lizard (*P. muralis*) in Germany. The quantity and quality of the isolated DNA collected by buccal swabbing and by screwing a tail tip of the same individuals was compared. Although the DNA yield from buccal swabs was much lower than from tissue, it was sufficient for a successful amplification. We genotyped the individuals at two microsatellite loci. Buccal swabs generated genotypes just as well as tissue samples. We could not find a lower threshold of DNA quantity that increased genotyping errors. In contrast, very high DNA yields (>10 ng/ml), as found in some tissue samples, produced a higher number of unspecific peaks. These results show that buccal swabs are a simple and efficient non-invasive sampling method for DNA analysis in adult lacertid lizards. Carefully applied, the technique does not harm the specimens in their locomotor performance and energy reserves. An additional advantage of buccal swabbing is the time-saving DNA extraction, since there is no need to remove scales, chop up the tissue, nor for a long digestion step.

Keywords: conservation genetics, DNA extraction, genotyping, microsatellite, non-invasive sampling, *Podarcis muralis*.

During recent decades, the availability and use of microsatellites as marker system for population genetic studies has strongly increased (Selkoe & Toonen 2006). Microsatellites are particularly suitable to gain insights into fine-scale processes in conservation genetics, such as inbreeding depression, hybridization and consequences of habitat fragmentation. Tissue sampling (toes and tail tips) is often considered the most reliable technique to gain DNA-templates for genetic analyses in amphibians and reptiles. Ethic concerns about animal welfare and conservation (May 2004, Funk et al. 2005) fostered the search for alternative non-invasive methods that do not harm individuals (Pidancier et al. 2003). Buccal swabbing has recently been suggested as a routine non-invasive technique to gain DNA-templates from amphibians (Broquet et al. 2007) and reptiles (Miller 2006).

Nevertheless, genetic analyses using buccal swabs are still very scarce, particularly among lacertid lizards (Beebee 2008). This is caused by the naturally high tail autotomy rates within lizard populations that make tail tips as tissue samples ethically justifiable. However, it is has been shown that caudal autotomy reduces arboreal locomotor performance in climbing lizard species, since the tail serves as a counterbalance (Brown et al. 1995). Furthermore, the tail serves as an important lipid energy reserve for hibernation and autotomy may, therefore, be energetically costly.

We tested the performance of buccal swabs for DNA analysis in an introduced population of the Common Wall Lizard (*P. muralis*) in Germany (Schulte et al. 2011). We compared the quantity and quality of the isolated DNA collected by buccal swabbing and by screwing a tail tip of the same individuals. We then genotyped the individuals at two randomly chosen microsatellite loci.

We sampled 49 adult lizards (Snout-Vent Length ≥ 50mm) from an introduced wall lizard population in Dresden, Saxony. Lizards were captured by hand or by noosing in August 2009. No lizard displayed autotomy while catching. Sampling of buccal cells was carried out using a diagnostic fine-tip dry swab (Medical Wire & Equipment, MW-100) by comprehensively swabbing each specimen underneath its tongue and cheek for about a minute. Nearly all individuals bit voluntarily into the swabs. Samples were stored in sterile tubes at -20°C until DNA extraction. DNA was extracted using the Qiagen DNEasy blood and tissue kit following the manufacturer's protocol (adding PBS buffer). For comparison, a small tail tip (5 mm length) of the same individuals was screwed until autotomy occurred. Individuals were immediately released after sampling. Tissue samples were stored in 99% ethanol p.a. at room temperature until DNA extraction. After removing scales, DNA was extracted from muscle tissue of

tail tips using the Qiagen DNEasy blood and tissue kit.

Total DNA yield obtained from swabs and tissue was quantified with a Qubit® Fluorometer (Invitrogen) and compared with a paired t-test. To test the reliability of DNA templates for microsatellite genotyping, we genotyped all specimens at two loci using primers specifically developed for *Podarcis muralis* (C8f: FAM-GACAATCCAATGTACAGAGCAAG, C8r: AACACACATGCACAAACCAC; B4f: HEX-AATCTGCAATTCTGGGATGC, B4r: AGAAG-CAGGGGATGCTACAG; Nembrini & Oppliger 2003). For the PCR reactions we used three different templates: i) DNA from tissue diluted 1:10 with ultrapure water, ii) undiluted DNA from swabs for the twelve samples with the lowest DNA yield and iii) swabs diluted 1:10 with ultrapure water. PCR amplification was performed in a 20 µl reaction volume containing 8 µL 5Prime HotMasterMix, 10 µl ultrapure water and 0.5 µl of the forward and reverse primers, respectively. PCR amplifications were performed in a Multigene Gradient Thermal Cycler (Labnet) using the following profile: initial denaturation of template DNA for 2 min at 94°C; 35 cycles for 30 s at 94°C, 50 s at 57°C and 45 s at 65°C; and a final extension of 3 min at 65°C. PCR products were genotyped on a MegaBACE 1000 automated sequencer (GE Healthcare). We used FragmentProfiler 1.2 (Amersham Biosciences) for scoring the data. We compared the results of allele lengths, the number of amplification failures and the occurrence of unspecific peaks between swab and tissue samples and between loci (χ^2 cross tabulation test). To test for differences in the number of unspecific peaks between loci, we used Fisher's Exact Test, as the expected value for one locus was smaller than five. Peak heights were compared among sampling techniques by calculating the mean peak height per individual and locus. We first performed an ANCOVA with the explanatory variables "locus" and "sampling method" and the covariate "DNA content". As we found a significant interaction between sampling method and DNA content, we tested for correlation between peak height and DNA content separately for both loci and sample methods using a linear regression model. A similar procedure was performed to evaluate whether the occurrence of unspecific peaks is correlated with the sampling method, locus or DNA content. Due to the binomial data format (unspecific peaks present = 1, not present = 0), we used a Generalized Linear Model with binomial error distribution. Afterwards, we simplified the model using the step function in R. All statistical analyses were performed in R 2.12.0 (R Development Core Team 2010).

The DNA yield obtained from tissue was significantly higher than from swabs of the same individuals (paired t-test, df = 48, t = 9.75, $p < 0.001$; Fig. 1). Scoring results were identical among swab and tissue samples for all individuals. Amplification failed significantly more often in locus C8 than in locus B4 (χ^2 cross tabulation test, $\chi^2 = 6.27$, df = 1, p = 0.01), but was not influenced by sampling method (B4: χ^2-test, $\chi^2 = 0.1$,, df = 1, p = 0.76; C8 = χ^2-test, $\chi^2 = 0.21$, df = 1, p = 0.65; Table 1).

We found a significant interaction between sampling method and DNA yield on peak height (ANCOVA, $F_{1,148} = 8.0$, p = 0.005; Table 1). As peak heights differed significantly between loci (ANCOVA, $F_{1,148} = 56.3$, $p < 0.001$) and sampling method ($F_{1,148} = 7.45$, p = 0.007), we analysed each locus and method separately. Peaks of tissue samples were significantly higher than peaks of buccal swabs for locus B4 (paired t-test, df = 38, t = 3.3, p = 0.002), but not for locus C8 (paired t-test, df = 24, t = 0.95, p = 0.35). Peak heights differed significantly between loci for both swab samples (paired t-test, df = 35, t = 3.7, $p < 0.001$) and tissue samples (paired t-test, df = 28, t = 6.2, $p < 0.001$). The number of unspecific peaks was significantly higher for locus C8 than for B4 (Fishers Exact-test; $\chi^2 = 17.3$,

Table 1: Genotyping success, average peak heights and number of unspecific peaks in swab and tissue samples (n = 49).

	swabs (diluted 1:10)	**tissue (diluted 1:10)**
primer C8		
number of genotyping failures	12	15
average peak height	1285.1 ± 211.5	904.5 ± 161.6
number of unspecific peaks	11	45
primer B4		
number of genotyping failures	7	5
average peak height	3273.9 ± 510.9	5828.1 ± 648
number of unspecific peaks	2	7

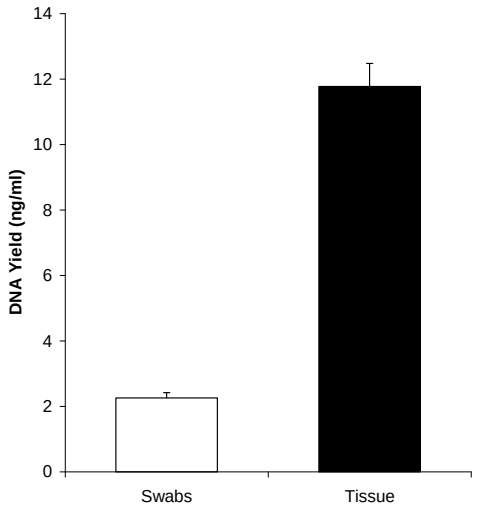

Figure 1: Total DNA yield (ng/ml) for buccal swab sampling and tissue sampling for the same individuals (n = 49). Error bars are standard errors.

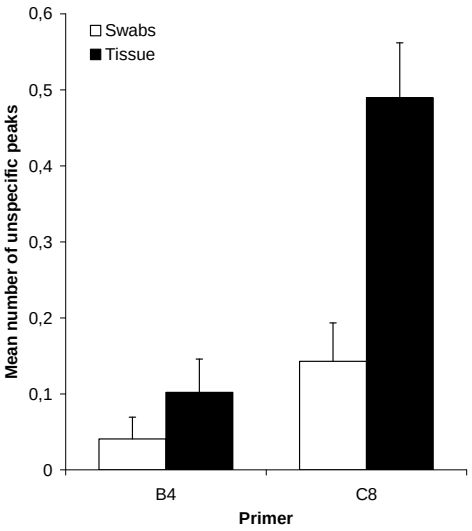

Figure 2: Mean number of unspecific peaks between loci and between swab and tissue samples (n = 49, respectively). Error bars are standard errors.

df = 1, p < 0.001; Fig. 2). Unspecific peaks occurred significantly more often in tissue samples than in swab samples for primer C8 (χ^2-test, $\chi^2 = 12.1$, df = 1, p-value < 0.001), but not for primer B4 (Fishers Exact-test, χ^2-test, $\chi^2 = 0.62$, df = 1, p = 0.43; Fig. 2).

In the swab data, we found a significant positive correlation between peak height and DNA yield for both loci (linear regression, B4: p = 0.001, $R^2 = 0.23$; C8: p = 0.04, $R^2 = 0.11$). This correlation was most obvious in the lower range of yield of DNA up to 3 ng/ml (Fig. 3). Large peak heights were already reached at 2 ng/ml. At 3-5 ng/ml DNA yield, peak heights were not increasing anymore. Peak heights < 40 were not distinguishable from background noise. For tissue samples, no significant correlation between peak height and DNA yield was found. The yield of DNA was not significantly correlated with amplification failures (ANOVA, $F_{1,192} = 0.24$, p = 0.62). We did not found a lower threshold of DNA yield for successful amplification, as even the lowest DNA yield (0.097 ng/ml) generated genotypes. Occurrence of unspecific peaks was significantly correlated with the yield of DNA in tissue samples for primer C8 (Generalized Linear Model: df: 190, z = 2.15, p = 0.03). 57.7% of samples with a DNA yield > 10 ng/ml exhibited unspecific peaks, whereas only 22% of samples with a yield of DNA < 10 ng/ml exhibited unspecific peaks.

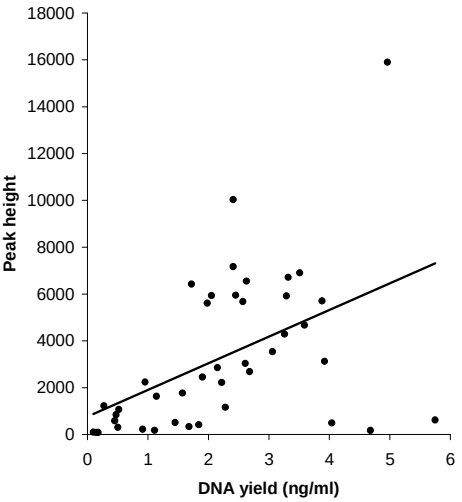

Figure 3: Correlation of DNA yield and peak height among swab samples for primer B4 (n = 49).

Our results corroborate other studies in identifying buccal swabbing as a reliable non-invasive tissue sampling method for DNA analysis in reptiles (Miller 2006, Beebee 2008). Buccal swabs generated genotypes just as well as tissue samples. However, in contrast to Beebee (2008) the results obtained from swabs were not more reliable than

those from tissue samples. Beebee (2008) suggested that the sampling technique of screwing tail tips might facilitate a higher probability in contamination of DNA in tissue samples. This can be avoided by a thorough and clean working procedure. Although the DNA yield from buccal swabs was much lower than from tissue, it was sufficient for a successful amplification. We did not find any lower threshold of DNA quantity that increased genotyping errors (Taberlet et al. 1999). In some cases the DNA yield from buccal swabs reached rather high values, but this was possibly caused by partial bleeding of some individuals. Interestingly, our results also show that very high DNA yields (>10 ng/ml), as found in some tissue samples, might be disadvantageous as they produce more unspecific peaks. In these cases, a dilution can help to optimize the results.

In conclusion, buccal swabs are a reliable non-invasive sampling method for DNA analysis in lacertid lizards that do not harm the specimens in their locomotory performance and energy reserves. Furthermore, this kind of sampling is probably less stressful for the species. However, patience and caution is needed to wait for the lizard to stop biting into the swab in order to preserve their teeth. It has to be mentioned that this sampling method can only be used for adult lizards with a minimum snout-vent length of 50mm, since even the smallest available swabs are too big for sampling smaller *P. muralis* without harming the individual. Whenever possible, flexible dry swabs with a fine narrow bud, like the MW-100 swab (Medical Wire & Equipment) should be used, since buds of standard cotton swabs are too big for sampling. Apart from the primary concern of the animals' welfare, another advantage of buccal swabbing is the time-saving DNA extraction, since there is no need to remove scales, chop up the tissue and also not for a long digestion step. Moreover, the method will certainly help to convince nature conservation authorities that sampling for population genetic studies will not harm individuals in natural populations.

Acknowledgements. This work benefited from a grant of the 'Deutsche Bundesstiftung Umwelt' (DBU) (no.: 27282/33/2). We are grateful to Petra Willems for help in the laboratory. Furthermore, we thank Richard Podloucky for suggestions and two anonymous reviewers for their helpful comments. Permits for sampling Wall Lizards were obtained by the responsible administration of the federal state of Saxony.

References

Beebee, T.J.C. (2008): Buccal swabbing as a source of DNA from squamate reptiles. Conservation Genetics 9: 1087-1088.

Broquet, T., Beset-Braendli, L., Emaresi, G., Fumagalli, L. (2007): Buccal swabs allow efficient and reliable microsatellite genotyping in amphibians. Conservation Genetics 8: 509-511.

Brown, R., Gist, D.H., Taylor, D.H. (1995): Effect of caudal autotomy on locomotor performance of wall lizards (*Podarcis muralis*). Journal of Herpetology 29: 98-105.

Funk, W.C., Donelly, M.A., Lips, K.R. (2005): Alternative views of amphibian toe-clipping. Nature 433: 193.

May, R.M. (2004): Ethics and amphibians. Nature 431: 403.

Miller, H.C. (2006): Cloacal and buccal swabs are a reliable source of DNA for microsatellite genotyping of reptiles. Conservation Genetics 7: 1001-1003.

Nembrini, M., Oppliger, A. (2003): Characterization of microsatellite loci in the wall lizard, *Podarcis muralis* (Sauria: Lacertidae). Molecular Ecology Notes 3: 123-124.

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

R Development Core Team (2010): R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Schulte, U., Hochkirch, A., Lötters, S., Rödder, D., Schweiger, S., Weimann, T., Veith, M. (2011): Cryptic niche conservatism among evolutionary lineages of an invasive lizard. Global Ecology and Biogeography 20: doi: 10.1111/j.1466-8238.2011.00665.x.

Selkoe, K.A., Toonen, R.J. (2006): Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9: 615-629.

Taberlet, P., Waits, L.P., Luikart, G. (1999): Noninvasive genetic sampling: look before you leap. Trends in Ecology and Evolution 14: 323-327.