Comments on the Collection Of Buccal Swabs From Amphibians

By Steven J. R. Allain

Minimally invasive DNA collection techniques are one of the marvels of the modern scientific age due to increased sensitivity and precision of the associated sequencing equipment. This is of course due to advances in technology and our growing understanding of the specific areas of science that deal with genetics and DNA. No longer do we need to collect tissue samples using questionable methods (which may make your institution's ethics board cringe). Recently I've been involved with a project with my old friend the Common Midwife Toad (*Alytes obstetricans*) here in the UK, in terms of collecting buccal samples in order to answer a very important question: where did they come from? As a non-native species we are interested in how many different introductions have been made and whether or not the toads are all the same species. We've already been swabbing them for disease as you may have read in previous issues and so far the news is good, the population I'm intimately investigating is free from chytrid!

Now back to the whole reason for this short comment, so far the genes within the mitochondrial genome have been sequenced from this population and compared with those in reference libraries such as GenBank; this has allowed myself and the rest of the team to slowly piece this puzzle together. It is going to take us another year or so to collect samples from the other known populations and build the complete picture. In the meantime I thought that I would just highlight some hurdles we've had along the way to help others completing similar studies in the future.

Buccal swabs have been an effective tool for collecting DNA from amphibians for quite some time (*1*), as I'm sure anyone who has seen CSI can agree. The previous widely used was that of toe-clipping but this wasn't always the most ethically responsible option and often had unwanted side effects (*2*, *3*) despite it's supposed minimal impact. Of course tissue samples are still a valuable source of DNA but I would urge to only collect these if necessary and preferably from deceased individuals. Thankfully technology has caught up and buccal swabs are the preferred technique which have been shown to be perfect for reliable microsatellite sequencing (*4*). This can in turn help with species delimitation, look at inbreeding within a population or as I am looking at the phylogeography of an introduced species from many multiple unknown sources.

Now comes the tricky part as you can't exactly ask the frog, toad or salamander in question to open its mouth and voluntarily give you a sample like in the TV crime dramas. Instead you have to forcibly open the little guy's mouth to collect the mucosal cells needed to complete the DNA analysis. Now this can be done safely in a number of ways but the easiest ways that I've found to do so are to either use a guitar pick, a disposable plastic spoon or similar instrument (*5*). Both of these are of course quite blunt and shouldn't cause any harm to the fragile oral cavity of the amphibian in your hand – their use is needed just to convince the subject to open its mouth so you can quickly get a sample and then release them at the point of capture. When swabbing it is vital that the 'cheeks' of the amphibian are swabbed as is the roof of the mouth. Do take care when working in this latter area as you may end up poking the poor amphibian in the eye from the inside of its oral cavity. Be sure to twirl the swab between your index finger and thumb between 10-12 times in each area to ensure enough cells are collected to provide a viable sample.

It is important to note at this point that if you're going to complete your own similar study that sterile gloves will need to be worn at all times. The swabs used by myself and others with such projects are Medial Wire's MW-100 dry swabs, which handily come packaged sterile and can be stored in a fridge or freezer until the time when the analysis is going to be completed. For more guidance please read the literature cited but I'm confident that I've covered most of the main problem areas that our volunteers have been having.

Photo: Hannah Parker.

References:

1. C. S. Goldber., M. E. Kaplan & C. R. Schwalbe. *Herpetol. Rev.* **34**, 220–221 (2003).
2. J. J. Van Gelder & H. Strijbosch. *Amphib. Reptil.* **17**, 169–174 (1996).
3. N. Golay & H. Durrer.. *Amphib. Reptil.* **15**, 81–83 (1994).
4. T. Broquet, L. Berset-Braendli, G. Emaresi & L. Fumagalli. *Conserv. Genet.* **8**, 509–511 (2007).
5. J. R. Poschadel & D. Möller. *Conserv. Genet.* **5**, 865–867 (2004).