Environmental DNA Analysis – Detection of Endangered and Invader Species in Wildlife Conservation

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The most non-invasive testing or confirmation of the presence of a species in a given environment can be accomplished by detection of the DNA they leave behind in the environment, be it by release of DNA directly into a soil or aquatic environment – like a lake or a pond (e.g. Thomsen et al 2012), or by leaving behind DNA-containing material such as hair or scat (e.g. Hansen & Jacobsen 1999).
In either case, DNA recovered will be trace amounts of degraded DNA and consequently requires highly sensitive methodology for detection and analysis. Aside from the trace level content of DNA and potentially high degree of degradation of DNA in these types of samples, the possibility of substrate-based inhibition of PCR-based assays has to be taken into consideration in both assay development and interpretation of results. In each case, the goal is to develop a highly sensitive assay, which is optimized for the detection of minute traces of highly degraded DNA in the presence of inhibiting substances.

Confirmation of the presence of species in aquatic environments by detection of environmental DNA in water samples
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The presence of a species in an aquatic environment – like a pond or lake – can be confirmed by detecting the DNA members of the species leave behind in the environment, by analysing DNA extracted directly from samples of the body of water (Ficetola et al. 2008, Jerde et al. 2011). Faeces, urine and epidermal cells are believed to be the predominant sources of environmental DNA (Lydolph et al. 2005; Haile et al. 2009), which may survive for up to two weeks after the species left the aquatic environment (Thomsen et al 2012). This trace DNA can be extracted from the water and detected utilizing species-specific primers in highly sensitive real time PCR assays. This was demonstrated for aquatic species like fish (e.g. loach *Misgurnus fossilis*), amphibia like newts (e.g. *Triturus cristatus*), frogs (e.g. *Rana crovalis, Bufo bufo*) as well as semi-aquatic mammals like the otter (*Lutra lutra*) and insects like the dragon fly (*Leucorrhinia* pectoralis) among others (Thomsen et al 2012).

In our project, we build on experience gained in validation and utilization of existing methodology and go beyond the previously published by developing new assays for species which so far have not been detected using this approach.
In an initial phase, we validated published methodology for confirmation of the presence of great crested newt (*Triturus cristatus*) in an aquatic environment by real-time PCR based detection of species specific DNA (Biggs et al 2014, Thomsen et al 2012), which is now utilized in environmental testing in the current newt breeding season. Building on the experience gained here, we will develop new real-time PCR based assays for further endangered species, as well as invader species, which may pose additional risk to endangered species native to the environment.

Non-invasive detection of terrestrial species by analysis of species-specific DNA in scat
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The second project focuses on terrestrial species and their detection by utilizing DNA traces that can be found in their droppings (scat):
Studies in the wildlife conservation context traditionally utilize observational survey techniques (e.g. O'Sullivan 1983), which can prove difficult to accomplish in case of species which are rare, nocturnal and elusive like the pine marten (*Martes martes*; e.g. Wilson & Delahay 2001). Consequently, a variety of approaches of DNA analysis-based methodology have been utilized to confirm the presence of the species in the environment for a number of years, by using non-invasively collected samples such as hair and scat (faeces). Scat samples contain traces of species-specific DNA due to accumulation of exfoliated epithelial cells from the intestinal wall of the defecating individual (Albaugh et al. 1992). DNA extracted from the sample material is analyzed either by direct sequencing of mtDNA (e.g. Murakami 2002), PCR-RFLP assays (e.g. Hansen & Jacobsen 1999) and real-time PCR based assays using species-specific primers (e.g. Beja-Pereira et al 2009). The critical requirement for any kind of assay based on non-invasive samples like scat is that it is sensitive enough to detect ttrace quantities of beyond that degraded DNA, as commonly recovered from this kind of material (Taberlet et al. 1999).

The goal of our study is the development of a species-specific assay, which is sensitive enough to allow for detection of pine marten (*Martes martes*) DNA from scat samples without the need for real-time PCR based detection, by designing species-specific primers targeting short amplicons located in genes like cytochrome b or cytochrome oxidase I and utilizing optimized methodology to neutralize enzyme inhibitory substances commonly present in this kind of sample material.

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