European Raptor Biomonitoring Facility
Advice Hub

Measuring genetic variation for contaminant analyses

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MEASURING GENETIC VARIATION FOR CONTAMINANT ANALYSES

The use of genetic data has found its way into in wide range of different disciplines, such as medicine, archeology, ecology, and forensic science. In many ways the use of genetic resources has revolutionized these fields. In biology, genetic information presently forms the basis for our understanding of the evolutionary processes behind population structuring, speciation, extinctions and natural selection at population level, as well as how genes influence the fitness of the individuals. Today, population genetics also forms an integral part of conservation biology.

GENETIC INFORMATION FOR CONTAMINANT MONITORING

Spatial and temporal variation of genetic diversity in natural populations has also become an important aspect of contaminant research. As chemical contaminants affect the survival and reproduction of the individuals, the contaminants will also affect the size and the demography of populations. As decreasing population size may by itself induce additional negative effects on the population, such as inbreeding, and increases the risk of (local) extinction, an understanding of the genetic diversity of various raptor species across Europe may help identify populations at higher risk. Low genetic diversity at the population level furthermore increases the population's susceptibility to e.g., environmental changes. Long time monitoring of these changes in population genetics can thus be seen as an integral part of a surveillance program.

GENETIC SAMPLES

From a live bird, blood is a good source for DNA. Taking a blood sample from a live bird, however, requires a lot of practice, and both legal and ethical permits are required for handling the bird as well as taking the sample. Also, the base of the feather shaft (rachis) may provide enough DNA for genetic analyses. However, feathers often yields very small amount of DNA and may not be suitable for all types of genetic analyses. It is important to note that plucking feathers from live birds also requires legal and ethical permits in most countries and that different rules may apply to different types of feathers

(for instance, for some species it may be possible to pluck a chest feather, but it is not allowed to pluck a flight or tail feather). A shed feather can also yield DNA for analysis, but as the DNA rapidly degrade when the feather is no longer attached to the body, this DNA is often of poor quality, and difficult and costly to work with. Therefore, a shed feather can be used if the genetic signature of a particular individual is important and no other genetic material is available, but as a regular way of obtaining material for large scale population genetic studies, shed feathers are not optimal.

Carcasses are a good source for DNA. DNA can be extracted from both soft tissue and bones. Liver, heart and muscle tissues are good sources and in particular the breast muscle is often large and easily accessible on a bird carcass. However, as the DNA rapidly degrades after death of the individual, the quality of the DNA will depend on how long the bird has been dead. The quality of the DNA is affected already hours after death, but several days old carcasses can still yield DNA with a quality that is suitable for most DNA studies. How fast the DNA will degrade, will depend on the humidity and the temperature in the place where the carcass has been laying. The DNA from a carcass that has been laying in cold and dry environment is often much better preserved than from a bird found in warm and wet conditions.

DNA can easily be amplified from very small amounts of tissue. This is a great advantage if only little material is available. However, this also makes DNA samples very sensitive for contamination with DNA from other individuals, so called cross-contamination. Even the smallest amount of tissue from another individual can interfere with and ruin subsequent genetic analyses. It is therefore very important that tissue that shall be used for DNA studies never come in contact with tissue containing DNA from another individual. Even the use of a scalpel blade that has been used for another individual can cause contamination and problems in later analyses. It is therefore very import to be very careful when taking tissue from a bird that shall be used for DNA studies.

STORAGE OF GENETIC SAMPLES

The sampled tissue should ideally be about 4-6 mm, preferably sliced so that the preservative (see below) reaches the inner parts of the tissue. Several similar-sized pieces can be taken to increase the total amount of material, but it is, however, necessary that the preservative, is at least 3-4 times the volume of the collected tissue. The sample can be stored in 2-20 ml plastic vials with screw lid (a type with o-ring reduces the risk of leakage). There is selection of different storage buffers that can be used. A range of alternatives have been proposed, e.g., 95% ethanol, DMSO-salt

solution, or Longmire lysis buffer. Several commercially available preservation buffers also exist.

DNA rapidly degrades at room temperature. Some preservative allow storage at room temperature for a limited period of time, but in order to ensure long time preservation it is essential to keep the samples as cool as possible. For longtime preservation cryogenic or -70° storage is optimal, but -20° is also acceptable. Even if the sample is stored in a preservative, a cool box with ice is an option for short time storage, in order to minimize the degradation of the DNA. Long-term storage in a freezer without a preservative is not recommended, as freezer will, sooner or later, break and if not detected immediately, the samples will be destroyed.

SHIPPING OF GENETIC SAMPLES

See the section on “shipping of genetic samples” for packing and information about legislations regarding transport of DNA samples.

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