Molecular markers in Corncrake biology

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Introduction

Molecular markers are a tool with growing applications in many fields of avian biology. Some molecular studies on birds represent milestones in the entire field of molecular systematics, e.g. the "tapestry"-phylogeny of all major bird taxa (Sibley et al. 1988). Besides phylogenetic applications that apply mainly at the taxonomic rank of species and higher, molecular markers can also reveal relationships among populations or even individuals. They can be used to address a number of population-based questions like geographic structure, genetic variability, migration rates and gene flow, as well as mating systems, paternity analysis, and sexing.

A couple of labs working with molecular tools in zoology have already conducted some initial studies on the possible use of molecular markers in corncrake research. But, to my knowledge, no results have been published so far. Hence, the intention of this contribution is not to review our current knowledge about the subject. Rather, a brief insight into the techniques and their potentials will be given as well as short instructions on how to collect and store samples for DNA analysis.

1. Source of DNA

DNA is part of any living cell. Theoretically, a single cell contains enough DNA to perform a PCR (polymerase chain reaction) amplification of a defined genetic marker. In practise, we have to choose the source of DNA according to the quantity of DNA we need. The less we know about a species' genome, the more DNA we need. Some techniques require larger quantities of DNA than others. Commonly used and very rich sources are body tissues, e.g. muscle or liver, and blood. At the other end of the scale are single feathers containing only tiny amounts of DNA. When working out the sampling plan one has also to bear in mind that some methods may cause a considerable amount of stress to the investigated animal or even kill it.

Feathers

Feathers are a convenient and easily accessible source but they offer only enough DNA for a few PCR amplifications. They are especially useful once a PCR assay has already been established, when only a few loci are being screened, and when a defined DNA fragment is being amplified. Freshly plucked feathers will usually yield more DNA of higher quality than old feathers found in the field. Exposure to the environment raises the risk of the feathers being contaminated with DNA from a foreign source. When taking feathers from a living bird, contour feathers would be preferred to remiges or tail feathers in order to minimise stress. For storage, the quill may be cut off using clean scissors or a razor blade. Clean forceps are recommended for handling.

Blood

Avian blood, unlike the blood of mammals, contains nucleated erythrocytes. The content of nuclear DNA is therefore very high. A drop of blood taken via a tiny puncture of the wing vein yields sufficient DNA for almost any DNA-based study. Before taking blood from living corncrakes in the field, the technique should be practised on corncrake-sized birds bred in captivity. It is important to use new instruments (needles, capillaries) for every sample to avoid cross-contamination.

Body tissue

Some tissues are a rich source of DNA, which is qualitatively and quantitatively comparable to blood. It has to be emphasised that there is no need to kill a bird to get DNA. But, when a bird is found dead, a piece of muscle, for example, can be used to extract large quantities of DNA. Unless the dead animal is frozen, soft tissues will contain DNA of high quality only for a short time. Once the autolythic process has progressed considerably, only skin and bones can be used to extract DNA although these tissues represent a comparatively poor source.

2. Storage and preservation

Any type of sample has to be properly labelled and documented. Apart from standard data (exact sampling site, date, collector's name etc.) notes on morphology, morphometrics and colour of the bird may be useful, e.g. for determination of sex or population specific markers. A second important point to consider is the danger of cross-contamination. Tiny, even invisible amounts of tissue can contaminate instruments, containers or reagents, which can subsequently lead to false PCR results.

Whole **feathers** are best stored in clean sealed plastic bags at room temperature or in a fridge. To save space, quills can be cut off and kept in microcentrifuge tubes. Alternatively, ethanol (concentration 75 % or higher) can be added to the quills.

Blood can be kept at ambient temperature when stored in a preservation buffer. A commonly used buffer component is EDTA (ethylene diamine tetra acetate) which prevents clotting and enzyme activity. In our preliminary study, we stored small amounts (a couple of microliters) of corncrake blood in 200 ìl of 0.25 molar EDTA, pH 8.0. Another preservative used to store avian blood is 25% DMSO (dimethylsulfoxide) saturated with NaCl (Arctander & Fjeldsa 1994). Alternatively, when no special preservative is available, ethanol (conc. 75 % or higher) may be used.

Fresh muscle **tissue** (app. 0.5 cm^3) should be stored in ethanol (conc. 75 % or higher) or frozen at –20°C in microcentrifuge tubes. A preservative suitable for both muscle tissue and blood is 25% DMSO saturated with NaCl. From museum skins or decayed animals pieces of dry skin can be taken. They should be stored like muscle tissue or, if no preservative is available, treated like feathers or feather quills.

3. Potential application of molecular markers in corncrake studies

Population studies

Commonly asked questions about corncrakes are those concerning populations. Are the fragmented populations in Western and Central Europe really self-sustainable or do they depend on the corncrake stock of Eastern Europe? Substantial numbers of corncrakes breed year after year in large agricultural complexes in Poland having no reproductive success at all. Where do the birds that are filling these population 'sinks' come from? In some areas shifts of corncrakes during the breeding season have been observed. Are these movements due to migration of certain geographic populations, or are the migrating birds just vagrants from anywhere in search of mating and breeding opportunities? In theory, answers to these questions could be obtained by radio tagging or extensive ringing and re-catching. In practice, however, such large-scale projects will hardly be feasible. Molecular markers offer an alternative. Potentially, marker systems can be developed that enable the identification of geographic populations. For single birds, the probability of belonging to a certain population may be determined.

Another set of questions concern the genetic structure of populations. Does the genetic structure of population correspond to their geographic distribution? Are there any parallels between morphological traits of corncrakes from different geographic areas and their genetic markers? How high are effective exchange rates of birds between populations measured in terms of gene flow? Are small and isolated populations subject to inbreeding? Is the differentiation among some population substantial so that they can be treated as separate genetic entities? These questions can be resolved by applying population genetic and molecular evolution models to the molecular data obtained. The answers will bring some new insight to important conservation issues.

Sexing

Corncrakes are well known for their difficult gender determination in the field. Morphology-based methods may perform well in certain geographic populations but may fail when the origin of the bird is unknown. Recently, a simple molecular technique for sexing of non-ratite birds has been developed (Ellegren 1996). The method uses the polymerase chain reaction (PCR) to amplify a gene encoding the chromo-helicase-DNA binding (CHD) protein. Male birds possess one copy of this gene, females, the heterogametic sex in birds, possess two. One of them is located on the W-chromosome, which is the female sex chromosome. PCR amplification of the CHD gene will result in one band in male birds and two bands in females. The primers developed by Ellegren (1996) seem to be universal for all non-ratite birds and thus probably will work also on corncrakes. Once optimised, this method would enable sexing of corncrakes in two to three hours. Other potential molecular gender determination methods, e.g. detection of W-chromosome-specific repetitive sequences or RAPD assay, are generally more time consuming.

Mating system

Many questions about the enigmatic mating behaviour of corncrakes have been resolved in the past decade with the aid of radiotelemetry. Some issues, however, have yet to be resolved, and molecular markers offer an appropriate tool: Are all males within a calling group contributing equal shares to the reproduction of a local population? Are some positions in calling groups more important than others and should they therefore be treated preferentially in terms of conservation management? How often does a male corncrake mate with different females? Does extra-pair mating play an important part in the reproduction of corncrakes? Answers to some of these questions will be of interest for the conservationist, especially in small and fragmented populations.

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