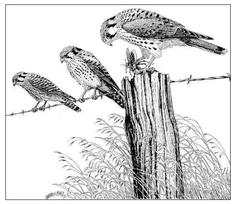

Use of RAPD-PCR for the genetic characterization of an American Kestrel (*Falco sparverius*) population in the mid Columbia River / lower Willamette River Drainage

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ABSTRACT

RAPD-PCR techniques were used to study the genetic diversity of a local population (n=12) of American Kestrels (*Falco sparverius*). Two RAPD primers were chosen to see which provided optimal homology and differentiation between individuals. Initial analysis using primer 2 (5'-d[GTTTCGCTCC]-3') indicated a common PCR product of approximately 280 bp present in all but one of the samples studied. Primer 3 (5'-d[GTAGACCCGT]-3') generated a PCR product (approx. 310 bp) common to all samples. Additional homologies were noted between >50% of the birds studied regardless of which primer was used. This initial data would suggest either a high level of relatedness between the birds studied or homology within the species. Additional research to include a larger sample from this population, representatives from other American Kestrel populations and statistical analysis of the data are still needed to provide a more accurate assessment of this method and of the diversity represented in this population.

INTRODUCTION

Genetic diversity within individual members of populations is necessary to maintain viable populations. Genetic diversity of sub-populations has ramifications in both ecological and evolutionary realms, with significant practical applications related to management of threatened and endangered species. Empirical data confirms that small isolated populations loose their genetic diversity as a result of drift (1). To document the degree of genetic diversity within a population, several methods have been employed. These include gene sequencing, DNA fingerprinting, analysis of protein polymorphisms, DNA-DNA hybridization and microsattelite polymorphisms.

In recent years, PCR based methodologies such as RAPD (random amplified polymorphic DNA) have been frequently used to study both interspecies and intraspecies diversity (2,3,4,5,6); including the establishment of paternity (7,8,9) and variations within populations. By enabling the detection of genomic polymorphisms at multiple loci, the RAPD markers generated have been shown to allow unique identification of individuals and diversity in a population through the analysis of banding patterns produced through agarose gel electrophoresis. For our work, this approach is of particular advantage over traditional DNA fingerprinting on three key points: 1) it eliminates the need for hybridizations involving radiolabeled probes and the laboratory and personnel safety requirments associated with those procedures, 2) it involves the use of short random primers (10-mers) so specific regions of allelic variation for study do not need to be identified and sequenced for the construction of specific primers for PCR (although RAPD primers will need to be screened to optimize banding patterns), 3) the commercial availability of self-contained reagent systems makes the process easily repeatable at a relatively low cost.

Our research was designed to provide insight into the natural history patterns and evolutionary aspects of a local population of American Kestrels (*Falco sparverius*) by determining which of a set of RAPD primers would yield the best differentiation of individuals. Further, we attempt to assess the genetic diversity of the local population of American Kestrels breeding within the mid-Columbia River / lower Willamette River drainage of Oregon and Washington.

Male American Kestrel

METHODS

Field Methods

Blood Samples were collected from American Kestrels trapped in Multnomah and Clackamas counties in Oregon and Clark County, Washington during the breeding seasons of 2004-2006. Adult birds were trapped via *bal-chatri* traps or within the nestboxes. All subjects were banded with U.S. Fish and Wildlife Service bands ensuring confirmed identification of individual birds and standard morphometric data was collected for all individuals (10). Blood samples (0.05 ml) were collected from the brachial vein. All samples were collected in accordance with *Guidelines to the use of wild birds in research* (11).

Sample Storage

After collection, blood samples were preserved by either of two methods: 1) 50µl whole blood diluted in 1.0 ml Queens lysis buffer (Seutin *et al*., 1991) or 4.0 µl whole blood diluted in 600 µl of Cell Lysis Solution (GenomicPrep Blood Isolation Kit, Amersham Biosciences). After collection and dilution, samples were stored frozen at -20^{0}C.

DNA Purification

DNA was purified using a commercial kit following the manufacturer's protocol for nucleated whole blood (GenomicPrep Blood DNA Isolation Kit, Amersham Biosciences). In addition, optional protocols for RNase A treatments were performed on cell lysates and samples were incubated on ice prior to the addition of "Protein Precipitation Solution" to enhance protein precipitation.

PCR Reactions

PCR was performed using commercially supplied Ready-To-Go RAPD Analysis Beads (Amersham Biosciences). After initial screening of the six 10-mer primers included with the kit, two primers were chosen for sample analysis: primer 2 – (5'-d[GTTTCGCTCC]-3'), primer 3 – (5'-d[GTAGACCCGT]-3'). Corresponding DNA fragments were amplified using a PTC-100 Programmable Thermocycler (MJ Research, Inc.) using the following cycling conditions: 1 cycle at 95^{0}C for 5 min followed by 45 cycles of 95^{0}C for 1 min, 36^{0}C for 1 min, 72^{0}C for 2 min. *E. coli* BL21 (DE3) and C1a DNA, also supplied with the kit, were used as positive controls.

Electrophoresis

DNA fragments were separated on 20 cm long agarose gels (2% w/v) made with 1x TBE buffer and containing 0.5 µg/ml ethidium bromide. Electrophoresis using 5 µl of PCR products was performed at a constant 150 volts (BIO-RAD 300X computer controlled power supply) for approximately 3 hours. Finished gels were photographed using a UVP ultraviolet transilluminator (model TS-15E) and a Polaroid Photo-Documentation Camera (FB-PDC-34) using a Tiffen 40.5mm deep yellow 15 filter.

RESULTS

PCR products were generated from individual blood samples of adult birds (n=12). Use of RAPD primer 2 revealed a common band of approximately 280 bp that was present in all but one of the samples examined (Fig. 1); though this band was very faint in sample 913. Other major groupings of PCR products were observed near 1300 bp, 600 bp and 250 bp.

The use of RAPD primer 3 resulted in the generation of a PCR product (approx. 310 bp) that was common to all samples (Fig. 2). Homology was also observed in additional PCR products of 50% or more of the samples; particularly bands at approximately 1100, 420, 400 and 380 bp.

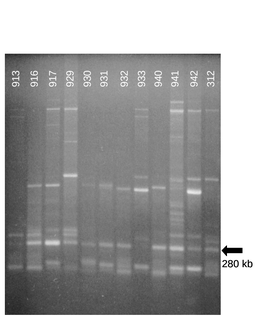

Figure 1. RAPD-PCR products using primer 2 (5'-d[GTTTCGCTCC]-3') from an American Kestrel population (n=12) taken from the mid-Columbia River / lower Willamette River drainage.

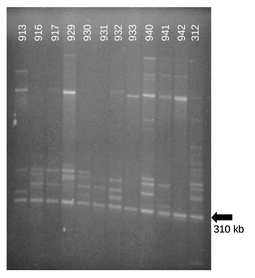

Figure 2. RAPD-PCR products using primer 3 (5'-d[GTAGACCCGT]-3') from an American Kestrel population (n=12) taken from the mid-Columbia River / lower Willamette River drainage.

DISCUSSION/CONCLUSION

Our preliminary results indicate that homology could be observed using either primer 2 or primer 3. However, significant variations within the population would require statistical analysis to effectively compare PCR products between individuals. Primer 3 does appear to provide a greater number of distinguishable bands showing greater homology between a larger number of individuals though this has not been verified statistically. It is unclear whether the observed homology could be due to either relatedness of individuals within the population or to overall species homology. To assess this we would need to include additional individuals from this population along with samples from other geographic populations and other kestrel species.

Clearly, further research needs to be conducted to evaluate the effectiveness of this RAPD-PCR method in making population generalizations. However, this method does show promise in terms of cost, reagent availability, ease of use and lack of radioactive materials.

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