E59 Paternity Testing and Next Generation Sequencing: New Approaches to Detecting Illegal Bird Trading

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Learning Overview: After attending this presentation, attendees will understand important aspects about wildlife trafficking in one of the most biodiverse countries, including its regulation, problems with the current form of identification of legal specimens, and new approaches using DNA to detect illegal bird trade.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing modern techniques applicable to wildlife forensics, especially in regard to illegal bird trafficking.

Birds born in captivity are identified by a small, individually numbered metal tag attached to the leg. However, despite many regulatory standards set for this identification, frauds are frequent and often hard to detect by conventional forensic methods. Paternity tests using microsatellites (Short Tandem Repeats in the DNA) presents an alternative for these cases.

Researchers from the Laboratory of Biotechnology and Molecular Markers developed a standardized paternity test for the blue-fronted Amazon (*Amazona aestiva*) using traditional methodology (Polymerase Chain Reactions (PCRs) followed by analysis of amplified fragments on a 3130 Genetic Analyzer with an estimated cost per sample of approximately USD $25.00) based on eight loci. This methodology, later employed in a joint inspection with the Brazilian environmental agency, successfully identified fraudulent bird trade, resulting in the shutdown of the breeding sites.

Building on the success of this approach, the same research group then focused on the development of a paternity test for *Saltator similis* (Green-winged Saltator) and *Sporophila maximiliani* (Great-billed Seed-finch), both listed as among the most trafficked species in Brazil.[1] This time, in order to develop a more cost-, time-, and labor-effective methodology, Next-Generation Sequencing (NGS) was utilized, which allowed the manipulation of 384 samples in a single run, representing a cost per sample of approximately USD $8.00. With this technique, microsatellites on contigs were found using msatcommander and the selected ones had their primers synthesized, PCR reactions standardized, and tested for their degree of polymorphism.[2]

For *Sporophila maximiliani*, ten selected loci were already tested in different populations and proved to be efficient in successfully detecting paternity. For *Saltator similis*, experiments are in the final stages and show promising results.

In summary, paternity testing is a promising technique to help enforcement agencies fight illegal bird trade. The use of NGS considerably facilitates this process, saving time and reducing costs.

Reference(s):

1. Destro, G.F.G. et al. Efforts to Combat Wild Animals Trafficking in Brazil. In: (Ed.). *Biodiversity Enrichment in a Diverse World.* INTECH Open Access Publisher, 2012. p.518.
2. Faircloth B.C. 2008. msatcommander: Detection of Microsatellite Repeat Arrays and Automated, Locus-Specific Primer Design. *Molecular Ecology Resources*, 8: 92–94.

Wildlife Traffic, Forensic Genetics, Next Generation Sequencing