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Skin From Feet of Museum Specimens as a Non-destructive Source of DNA for Avian Genotyping

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The advent of the polymerase chain reaction (PCR) has revolutionized sampling possibilities in avian genetic studies. With PCR, many genetic markers of interest can be amplified from samples such as single feathers (Woodruff 1990, Taberlet and Bouvet 1991, Morin et al. 1994, Srikwan and Woodruff 1996) and museum bird specimens (Cooper et al. 1992), which contain minute quantities of DNA and/or highly degraded DNA. The potential of using museum specimens in particular has opened up new avenues for phylogenetic and population genetic research in birds, which are only just beginning to be exploited (Smith et al. 1991, Cooper et al. 1992, Cooper 1994, Morin and Woodruff 1996). Museum collections are now seen as valuable repositories of genetic material (Graves and Braun 1992), and requests to curators for the use of museum specimens for genetic research are growing. However, obtaining a sample for genetic analysis from a museum skin necessarily involves removing part of the specimen, and there is great concern that damage to specimens be kept to a minimum. Previous authors have described the use of small pieces of skin from the body (Smith et al. 1991); single remiges or rectrices (Ellegren 1991, Leeton et al. 1993); or pieces of muscle, tendon, and bone (Cooper et al. 1992). Here, we report on the use of small pieces of skin from the soles of the feet of museum specimens used in the context of a population genetics study of the Loggerhead Shrike (*Lanius ludovicianus*). Because the sole of the foot has not to our knowledge been used as a taxonomic character in birds, the damage done to the specimens for future research is negligible. Furthermore, because we successfully analyzed single-locus nuclear markers (microsatellites) with these samples, few genetic questions exist that cannot be resolved with this tissue.

Methods.—With a sterile scalpel blade, pieces of skin approximately 1.5 × 1.5 × 3 mm were cut from the ventral side of the proximal phalanx of the first digit of the feet from 19 specimens of the San Clemente Loggerhead Shrike (*L. ludovicianus mearnsi*) that

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were obtained in 1915 and housed in two collections, the San Diego Natural History Museum (SDNHM) and the University of California, Los Angeles (UCLA). In addition, single flank feathers (approximately 3 cm long) with attached skin were plucked from the four specimens at SDNHM. Skin and feather samples were placed singly into sterile plastic envelopes. DNA extractions were performed in a UV hood with a commercial kit (QiAmp, Qiagen). About one-third of the foot-skin samples, or the whole feather base (calamus), was used in each extraction. Negative extraction controls, using the same instruments and reagents, were carried out simultaneously. Briefly, the tissue was digested in 180μL buffer ATL/20μL proteinase K solution for 20 hours at 55°C; other reagents and the spin column were used according to the manufacturer's instructions ("Tissue protocol"), and final DNA elution was performed with 2 × 100μL of 10mM Tris-HCl pH 9.0 preheated to 70°C. PCR reactions and direct double-stranded sequencing with ^{35}S-dATP were performed according to Mundy et al. (1996a), and microsatellite procedures followed Mundy and Woodruff (1996), except that the annealing temperature in all PCR reactions was reduced to 45°C, and the Mg^{2+} concentration was 3mM. Primer pairs used were DLL1/DLH1 for the mitochondrial control region (Mundy et al. 1996b) and LS1F/LS1R, LS2F/LS2R, LS3F2/LS3R2, LS4F/LS4R2, LTMR7F/LTMR7R, SJR4F/SJR4R (Mundy and Woodruff 1996), and STG4A/STG4B (Ellegren 1992) for nuclear microsatellite loci.

Results.—A 250 base-pair segment of the mitochondrial control region was successfully amplified from the foot skin of all 19 specimens, and for each specimen at least 200 base-pairs of sequence was obtained from two independent amplifications, mostly from separate extractions (Table 1). In contrast, a PCR product was obtained from only one of the four extractions from feathers, and this product was too weak to sequence directly. All seven nuclear microsatellite loci were successfully amplified and scored from DNA extracted from the foot skin of the 19 specimens (Fig. 1). No positive PCR results were obtained with negative extraction controls. More than 90% of the results have been duplicated, with independent extractions. When the microsatellite genotypes of the 19 individuals are compared over the seven loci, only two individuals have the same genotype.

Discussion.—Caution is required in interpreting data obtained from museum specimens, because the introduction of foreign DNA from a variety of sources (e.g. surface contamination on the specimens in the museum or contamination from other specimens in the lab) is a major concern. There are several reasons why we are confident that the present results cannot be attributed to contamination: (1) negative extraction controls were always negative, (2) PCR product was obtained from most samples in most reactions, (3) results were repeatable, and (4) different genotypes

TABLE 1. Example of mitochondrial control region sequence obtained from museum specimens of the Loggerhead Shrike. Haplotype "C" and numbering are from Mundy et al. (1996b), in which this segment of the Loggerhead Shrike control region sequence is validated. The other two sequences were obtained from independent extractions of specimen UCLA 9595. Dots represent bases identical to first sequence.

	Control region sequence
	245
Haplotype "C"	TGGTAACCTTCTAGGCACATTCCTATTTCAAGCACAATAAACCCAAGTCATCCTACCTGATAACACAAGACA
UCLA 9595	NNNNNN.....C..
UCLA 9595'	...........C..
	317
Haplotype "C"	AGCGTCACCCGAGGTCGGCAATGTTTACCTATGCATACCACAGTCCAGTAAACGAGGAATATCCTAGTACAT
UCLA 9595	..................T...
UCLA 9595'	..................T...
	389
Haplotype "C"	ACATGAATGCACTCGCCCATATGTTCCAGTCCACCTCCACAGACCCAATTCTCCACCAATGGCCTTCAGG
UCLA 9595	....
UCLA 9595'	....NNNNNNNNNNNNN

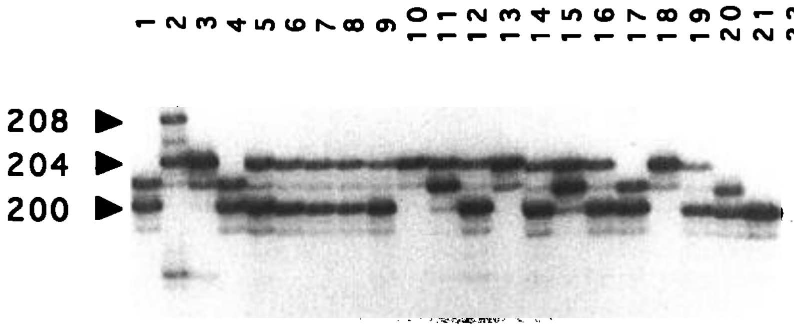

Fig. 1. Microsatellite locus LS4 amplified from museum specimens of the Loggerhead Shrike. Autoradiogram of PCR product generated with primers LS4F/LS4R2 and separated on a polyacrylamide gel. Each shrike sample shows one (homozygote) or two (heterozygote) strong bands of sizes 200, 202, 204, 206, or 208 base pairs; fainter bands are artifactual stutter bands generated in the PCR. Lanes 1 and 2 are two contemporary individuals of *L. l. gambeli* from San Diego, California, used as size markers. Lanes 3 to 21 are the 19 museum specimens of *L. l. mearnsi* included in this study. Lane 22, is the control extraction for museum specimens, which has not generated any PCR product. Genotypes and specimen numbers of museum specimens are as follows: 1, 200/202; 2, 204/208; 3 (UCLA 9595), 204/204; 4 (UCLA 9676), 200/202; 5 (UCLA 9585), 200/204; 6 (UCLA 395), 200/204; 7 (SDNHM 33117), 200/204; 8 (SDNHM 33118), 200/204; 9 (SDNHM 33119), 200/204; 10 (SDNHM 33120), 204/204; 11 (UCLA 377), 202/204; 12 (UCLA 378), 200/204; 13 (UCLA 415), 204/204; 14 (UCLA 416), 200/204; 15 (UCLA 424), 202/204; 16 (UCLA 9584), 200/204; 17 (UCLA 9593), 200/202; 18 (UCLA 9594), 204/204; 19 (UCLA 9633), 200/204; 20 (UCLA 9634), 200/202; and 21 (UCLA 9674), 200/200.

were obtained from different individuals. It is striking that the nuclear microsatellite loci were so readily amplified from our samples, because these loci are present as a single copy in the genome and are far less abundant than the nuclear 18S ribosomal genes and mitochondrial genes that have been the subject of most reports of DNA amplification from avian museum skins (e.g. Cooper et al. 1992, Smith et al. 1991, Leeton et al. 1993, Cooper 1994). During preparation of museum skins the feet generally are given no special attention, whereas in the past the skin was treated with one or more of a variety of chemicals, typically arsenic. This practice may result in less degraded DNA and/or lower concentrations of PCR inhibitors in the feet compared with the rest of the specimen. Our limited data from comparisons between feather and foot-skin extractions from the same specimens support this view. In conclusion, foot skin as a source of DNA for PCR amplification provides a convenient and minimally destructive sample that leaves the museum specimen essentially intact for future morphological study.

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Influence of Hatch Date versus Maternal and Genetic Effects on Growth of Black Brant Goslings

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Size of goslings at the end of their first summer is an important determinant of their fitness (Cooch et al. 1991a, Sedinger et al. 1995) because size influences first-year survival (Owen and Black 1989, Sedinger et al. 1995), size as adults (Cooch et al. 1991a, Larsson and Forslund 1991, Sedinger et al. 1995), and fecundity (Sedinger et al. 1995). Size of goslings is strongly associated with their hatch date, because late-hatching goslings grow more slowly than those hatching earlier (Cooch et al. 1991a, Sedinger and Flint 1991, Larsson and Forslund 1992, Lindholm et al. 1994).

Slower growth by late-hatching goslings has been attributed to poor foraging conditions experienced by these goslings, which is associated with the typical seasonal decline of nutrient levels in tundra plants eaten by geese (Sedinger and Raveling 1986) or reduced food abundance owing to grazing (Sedinger and Flint 1991, B. Person unpubl. data). Cooch et al. (1991a) controlled for genetic effects on growth by examining goslings from the same females nesting on different dates among years, or in later years during a long-term decline in growth (Cooch et al. 1991b). Other studies, however, have been unable to exclude the possibility that parental quality, or genetic or maternal effects, covaried with hatch date. If poorer-quality phenotypes or genotypes nest later, then late-hatching goslings may grow more slowly because they represent inferior genotypes, the eggs they hatched from were of poor quality, or they had poor-quality parents. We experimentally delayed hatching dates of Black Brant (*Branta bernicla nigricans*; hereafter "Brant") eggs to test influences of genetic and maternal effects on gosling growth.

Methods.—We removed the first egg from Brant nests and held them at ambient temperature for one to three days during the egg-laying period in 1991-1993. These eggs were then placed into nests containing one egg. To ensure that experimental eggs hatched synchronously with their foster siblings, it was necessary to delay only first eggs and to transfer these eggs into nests containing single eggs because Brant females begin incubation after laying their second egg (Flint et al. 1994). Switched eggs, therefore, hatched one to three days ($\bar{x}$ = 2 days) later than they would have if not switched. We compared growth rates of goslings hatching from delayed eggs with growth rates of goslings hatching naturally on the

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