

Museum Collections as Sources of Genetic Data

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Abstract. Museum collections are an under-utilized source of genetic material for avian systematics. Museum specimens are particularly valuable when the collection of new material is difficult or impossible. This is the case for rare or extinct birds and for regions where collecting is not allowed. Museum collections also provide opportunities for the study of recent evolutionary change. First, we illustrate the importance of voucher specimens for tissue samples collected for molecular systematics studies, reporting on several cases in which errors in the original identification were corrected after genetic data prompted a re-examination of the voucher specimen. Second, we report results from our molecular systematics studies of finches Viduidae and Estrildidae and cuckoos Cuculidae in which older museum specimens provided the genetic samples for many taxa. Finally, we discuss the use of feathers taken from museum specimens in molecular studies. Working with older feathers rather than fresh tissues entails additional work in the laboratory but has advantages in identification and repeatability, and museum skins are often the only material available for less-common species. The length of PCR product that can be amplified from feather extracts declines with specimen age, such that genes must often be sequenced in smaller segments (e.g., 200-300 base pairs, bp) in specimens that are 30 to 50 years old. We have consistently obtained mitochondrial DNA sequence data from specimens dating back to 1950 and have had success with a limited number of older specimens. Although the probability of success in amplifying a target gene declines with age, some skins in any age class may yield usable products for sequencing (the "universal primers" that often are used for cytochrome-b may be less useful than specific primers). Our success in amplifying and sequencing genes from museum skins encourages the use of collections for sampling critical specimens. The management of scientific resources includes both archival safekeeping of specimens collected from the field and the considered use of specimens in systematic research. We suggest that the association of genetic data gives specimens "value added" status and urge research museums to consider the benefits in addition to the costs of using specimens as primary sources of comparative genetic material.

Key words. ornithological collections, DNA, phylogeny, voucher specimens

1. DNA FROM MUSEUM SPECIMENS

Both archival and scientific use require the wise allocation of valuable museum specimens. After an initial enthusiasm for recovery of DNA from museum specimens (HOUDE & BRAUN 1988), museum curators realized there is a potential conflict between archival maintenance and progressive management of a scientific resource (GRAVES & BRAUN 1992). With their term „destructive sampling“ for the use of skin and feathers from museum specimens, GRAVES & BRAUN (1992) demand the researcher to justify the need for sampling an irreplaceable specimen. At the same time they recognize their importance for species that otherwise are unavailable for research. In response to increased interest in sampling specimens for genetic research, many research museums have established guidelines for sampling and criteria for museum curators to evaluate these proposals (USNM, FMNH, UMMZ). An alternate term for the activity is „value added sampling“ because the information acquired about a specimen's genotype contributes to the growth of scientific knowledge and the potential to solve biological problems. The specimens gain in value to our scientific community when they are used in this manner.

Using bird skins in museums as a primary source of genetic information has a number of additional advantages. Skins are a potential source of genetic samples for birds that are not possible to collect in the field, due to extinctions or political considerations. Museum specimens also can be used to test repeatability (a second sample can be taken from a known single source), can be examined to verify identification (RUEDAS et al. 2000) and to provide new information as molecular techniques develop and new biological questions arise.

Of course, material that is collected in the field specifically for genetic analysis is generally of higher quality and utility than material that can be recovered only with much labor from museum specimens (ARC-TANDER & FJELDSÅ 1994). Tissue samples for genetic analysis should always be saved when a bird is collected and a specimen is prepared. Muscle tissue or blood can be preserved in liquid nitrogen (LN2) or in suitable buffer (SEUTIN et al. 1991), or even in ethanol. A well-designed research program includes preserved samples of both blood and muscle tissue and a specimen, both as a voucher for identification purposes and for its value in augmenting existing natural

history collections apart from its immediate application to a particular genetic study. The cost in money and time required to obtain permits for scientific work is about the same for trapping birds and collecting only genetic samples as it is for collecting complete specimens. As the scientific value is greater for the complete sample, we encourage molecular systematists to secure their own genetic samples in the field and to collect new museum specimens for systematic research (REMSEN 1995).

Nevertheless it is not always possible to get fresh material for genetic studies. Moreover, certain evolutionary questions involve sampling museum specimens to test evolutionary change through time (e.g., THOMAS et al. 1990; GLENN et al. 1999) and to identify historically important material such as type specimens (PRINZINGER et al. 1997). Little work has been done with older specimens, because DNA degrades with age or because the skins were preserved with chemicals such as arsenic (PÄÄBO 1990; ELLEGREN 1994). The past decade has made increasing use of PCR amplification to obtain sequences from minute fossil or forensic amounts. The quality of preserved material necessary to do this work is not nearly as restrictive as the liquid nitrogen-preserved tissues generally used in restriction site analysis (HILLIS et al. 1996; KLEIN & PAYNE 1998).

DNA can be extracted, amplified and sequenced from museum study skins and skeletons both for phylogenetic analysis and for population-level work with microsatellites (e.g. TABERLET & BOUVET 1991; ELLEGREN 1992; LEETON et al. 1993; COOPER 1994; COOPER et al. 1996, 2001; MUNDY et al. 1997; PRINZINGER et al. 1997; ENGSTROM et al. 1999; SORENSON et al. 1999; CRACRAFT & FEINSTEIN 2000; DUMBACHER & FLEISCHER 2001; SEFC et al. 2001, 2002; PAYNE et al. 2002). Recently collected skins are more useful than older skins (GLENN et al. 1999). Our research with finches and cuckoos has had high success in recovering genetic sequence data from specimens collected back to 1900, but success in recovering data from the earlier specimens (e.g., < 1960) required amplification of relatively short PCR fragments (e.g., < 200-300 base pairs in length). In other laboratories, genetic information has been recovered from bird skins collected as long ago as 1860 and 1874 (ELLEGREN 1992, 1994; PRINZINGER et al. 1997).

Birds collected in recent years are often taken specifically for preserved tissues to be used in genetic studies. In many studies, voucher specimens (skins or skeletons, or both) are retained to support the identification; a multiple preparation is most useful (WINKER 2000). Specimens preserved in alcohol can be vouchers for later identification, but specimens preserved in formalin are less useful for genetic analyses because of the difficulty of extracting and amplifying DNA. Recovery of genetic sequence is more difficult when skeletons are cleaned of all connective tissue and muscle. Retaining some connective tissue on a skeleton makes the specimen more useful both for anatomical research and genetic analysis.

For molecular genetics work we prefer to use muscle tissue that was preserved in the field. When fresh tissue is not available, we prefer feather samples over blood to avoid the risk of sampling nuclear copies of mitochondrial genes (SORENSON & QUINN 1998). Nuclear copies of mtDNA sequences (or 'numts'; Lopez et al. 1994) result from ancient transpositions of mitochondrial sequences into the nuclear genome. Over time, the mitochondrial and numt sequences diverge and evolve at the different rates of molecular evolution characteristic of the two genomes. In some cases, a numt resulting from an ancient transposition event is present in a group of related species descended from a common ancestor (e.g., ARCTANDER 1995; BENSASSON et al. 2001), while in others multiple transposition events have occurred within a single clade (SORENSON & FLEISCHER 1996). In either case, numts are a potential source of confusion in comparative analysis of mtDNA, the genome that has been used most widely in avian molecular systematics. The high ratio of mtDNA to nuclear DNA in muscle and feathers makes these tissues preferable to blood when mitochondrial genes are of interest (SORENSON & QUINN 1998). Feathers are a good source of mtDNA. A feather sampled from a museum skin can be

Tab. 1: Table of success (or sequence length recovered) vs age of specimen

Age of specimens	# samples extracted	# samples sequenced	# yielding PCR products <500 bp in length
Cuckoos			
1930 - 1939	1	1	0
1940 - 1949	2	2	1
1950 - 1959	16	14	8
1960 - 1969	30	26	17
1970 - 1979	6	6	6
1980 - 1989	11	9	8
1990 - 2001	18	18	18
Estrildidae			
1950 - 1959	5	5	5
1960 - 1969	15	13	13
1970 - 1979	9	9	9
1980 - 1989	6	4	4
1990 - 2001	45	44	44

replaced after the base of the quill is sampled, much as feathers dropped in the preparation of a study skin. Careful selection of a feather or feathers to sample (e.g., a contour feather from the back of the bird or an underwing covert or axillary) results in little or no effect on the specimen's appearance and negligible damage to the specimen. Because toepad morphology is potentially informative about avian relationships and adaptation (CLARK 1973), removing a toepad for genetic analysis is not necessarily less destructive to a specimen than removing a feather.

Costs for the museum collection as a primary source of genetic material are twofold:

1. Damage to specimens. Museum curators can determine whether specimens that are sampled will have significant value added, or whether the cost in terms of damage or even loss of specimens is greater than the expected scientific interest.
2. Time and effort. Museum curators can negotiate whether the museum or the geneticist is responsible for removal of the feather, snip of skin, or toepad. The museum curator may be more conservative in selecting the sample, whereas the geneticist may be interested in obtaining sufficient material to carry out the molecular work with success. Taking very small samples is false economy if the sample yields no genetic data as a result.

2. GENETIC ANALYSIS OF MUSEUM SPECIMENS RESOLVES PHYLOGENETIC QUESTIONS

Our results in the analysis of mtDNA in feathers from museum specimens include several that are of interest to avian systematics and emphasize the importance of skins as primary sources of genetic information. Results include the following: African *Pholidornis ruficauda* is more closely related to the African warblers than to the penduline tits or the estrildids (SEFC et al. 2002); a hybrid *Vidua* (PAYNE 1980) indigobird x paradise whydah had an indigobird as the maternal parent (PAYNE & SORENSON, in prep.); drongo cuckoos *Surniculus* are related to *Cuculus* rather than to koels *Eudynamis*; New Guinea white-crowned black cuckoo *Caliechthrus* is closely related to *Cacomantis* and not to koels *Eudynamis*; and long-billed cuckoo *Rhamphomantis longirostris* is a *Chrysococcyx* (Sorenson & Payne, in prep.). Other results are mentioned in more detail:

Anomalospiza

The brood-parasitic cuckoo-finch *Anomalospiza imberbis* was once thought to be a ploceid finch, as its plumage is like that of weavers *Ploceus* and bishops *Euplectes* (SIBLEY & MONROE 1990). Its relationships are of interest because *Anomalospiza* and the indigo-birds and whydahs *Vidua* are the only Old World brood-parasitic songbirds. Understanding the relationships of *Anomalospiza*, *Vidua*, and their nesting relatives is needed to test ideas about the evolution of brood parasitism (PAYNE 1998). *Anomalospiza* feathers were sampled from UMMZ museum skins collected in 1968 and 1972 and processed in standard extraction, amplification and sequencing methods. Nucleotide sequences were compared with those of ploceid, estrildid and viduid finches and with other songbirds. *Anomalospiza* is most closely related to *Vidua*, and these two genera of brood-parasitic finches form a lineage that is sister to the estrildid finches Estrildidae, the group that includes the host species of *Vidua*. Brood parasitism evolved only once in the Old World songbirds, and the common ancestor of *Anomalospiza* and *Vidua* dates to perhaps as long as 20 million years ago (SORENSON & PAYNE 2001).

Estrildidae

The estrildid finches Estrildidae comprise about 140 species. Biological questions in the group include reconstruction of their biogeographic history with multiple dispersal events between Australasia and Africa, and relationships among the estrildid genera that are parasitized by *Vidua* finches, brood parasites that have frequently colonized new host species within an estrildid genus (KLEIN & PAYNE 1998). In addition, the relationships of species within the Estrildidae are not well known. Specimens were obtained by collecting tissues and feathers in the field, and by sampling feathers from museum specimens and live birds. For 33 of these estrildid species the source was a feather from a museum specimen (FMNH, MNHN, UMMZ, ZMFK, ZMUC).

Paludipasser locust finch

A feather resolved the evolutionary status of African locust finch *Paludipasser locustella*. The bird is basal to all other estrildid finches and it is not a waxbill (Estrildini). It was first described as a distinct genus *Paludipasser* by NAEVE 1909, then was placed with

quail-finch *Ortygospiza* by LYNES & SCLATER (1934). Locust finch resembles quail-finch *Ortygospiza atricollis* in having barred flanks in the female, and a red bill. CHAPIN (1954) noted, „in recent years these have usually been treated as members of a single genus, *Ortygospiza*, a course here followed with some reluctance... The beak of *P. locustella* is much deeper, with culmen more strongly ridged, and it actually shows a certain resemblance to that of *Anomalospiza*. The wings of *locustella* seem to me much smaller, proportionally, and its powers of flight not nearly so great. The legs, on the other hand, are exceptionally stout and muscular in *locustella*, more so than in any other estrildine finch I have dissected. The form of the gape wattles and the palatal spotting are still unknown, so I suspect we have more to learn of the relationships of *locustella*.“

The palate of nestling locust finch *Paludipasser* has an arc-shaped black line and a pair of short black lines behind the arc and at an angle to it. The gape has two flat red lobes and the lining of the mouth is bright red (IRWIN 1958). A study skin of a juvenile (FMNH 283598) was examined by softening the head in water. The melanin palate marks and gape lobes were visible as described by IRWIN (1958) though lacking the red colors. The palate of most waxbills has spots, whereas the palate of grassfinches (Poephilini) and munias and mannikins (Lonchurini) has lines (IMMELMANN et al. 1965,1977; RESTALL 1997). In contrast to locust finch, nestling quail-finch *Ortygospiza* has six palate spots, the palate is whitish, and the gape has three pale blue globes separated by black in a checkerboard pattern. The palate in locust finch is unlike the waxbills and is more like the munias and mannikins. The plumage pattern of barred flanks in locust finch occurs in several estrildid finches, not only in waxbills (quail-finch *Ortygospiza atricollis*, green avadavat *Amandava formosa*, goldbreast *A. subflava*) but also in Australian grassfinches (plum-headed finch *Aidemosyne modesta*, juvenile diamond firetail *Stagonopleura guttata*) and several munias and mannikins (including

chestnut-breasted mannikin *Lonchura castaneothorax*). *Paludipasser* is not closely related to quail-finch, to other waxbills, or to another group of estrildid finches. It is an estrildid finch with no close relationship to other finches (SORENSON & PAYNE 2001b).

Lonchura munias

Results with the munia *Lonchura* species are incomplete, but preliminary results show the validity of genetic sequences recovered from older museum specimens and the hybrid origin of a recently described bird, the cream-bellied munia *Lonchura pallidiventer*. The phylogenetic estimates of relationships within *Lonchura* are consistent with those of BAPTISTA et al. (1999). GOODWIN (1982), RESTALL (1997) and BAPTISTA et al. (1999) all recommended including „*Padda*“ *oryzivora* and „*P.*“ *fuscata* in the genus *Lonchura*, consistent with our results that also include *L. leucosticta* and *L. tristissima* in this clade (Fig. 1).

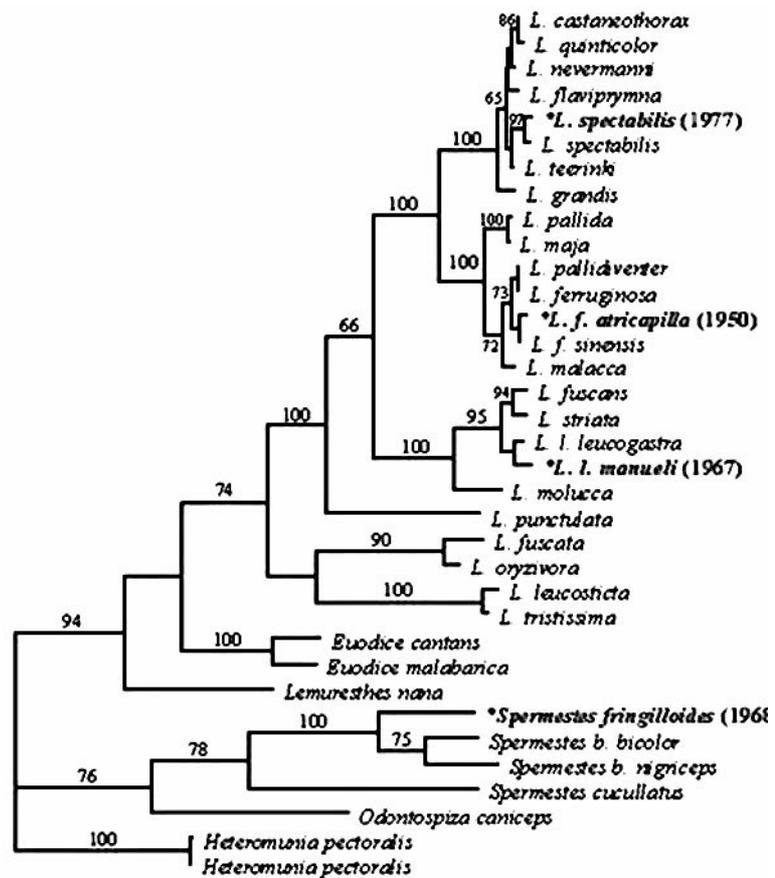


Fig. 1: Phylogenetic relationships of certain munia finches. The birds in boldface were sequenced from museum skins, with the date of collection indicated.

Cream-bellied munia *Lonchura pallidiventer* was described as a species from a bird market in Jakarta (RESTALL 1996), the birds said to be from southern Borneo. The bird has not been seen in the field and is thought to be a hybrid (VAN BALEN 1998). LUIS BAPTISTA provided feathers of two live birds that he obtained from ROBIN RESTALL through the San Diego Zoo. The bird was nearly identical in mtDNA sequence to chestnut munia *Lonchura (ferruginosa) atricapilla* (1 bp different) and tricolored munia *L. malacca* (3 bp different). The lack of difference from these munias suggests that *L. „pallidiventer“* is a hybrid, with the maternal parent *L. (f.) atricapilla*. The mitochondrial gene is transmitted maternally, so the hybrid would have the mtDNA of its mother. Nuclear genes are needed to determine the father by molecular methods. VAN BALEN (1998) suggested a hybrid origin with the parent species scaly-breasted munia *L. punctulata* and white-bellied munia *L. leucogastra*. Nevertheless, RESTALL (1997) described the song of „*pallidiventer*“ as like the song of five-colored munia *L. quinticolor*. Because munias learn their song from their father (GÜTTINGER 1973; CLAYTON 1989), *L. quinticolor* was probably the father of the hybrid.

L. „pallidiventer“ has a scaly feather pattern on the flanks. The scaly pattern occurs in several species of munia though not in *L. ferruginosa atricapilla* and *L. quinticolor*. The pattern appears occasionally in *L. malacca*, which is an allospecies with *L. ferruginosa atricapilla* (RESTALL 1997). RESTALL (1997) illustrates several munia species that regularly have a scaly pattern of feathers or bars on the flanks, and other species that sometimes have these patterns. The scaly gene is found in many species that lack the pattern but is expressed in hybrids even those involving the silverbills *L. castaneothorax*, *Euodice malabarica* and *E. cantans* (BAPTISTA, in litt., 1 July 1998). RESTALL (1997) observed nestbuilding in his captive „*pallidiventer*“ but had no young, and BAPTISTA had a pair of „*pallidiventer*“ nest and lay but the eggs did not hatch. Hybrids are known for many estrildid species, including some in different genera and tribes and some intergeneric estrildid hybrids are even fertile (IMMELMANN et al. 1977; FEHRER 1993).

3. THE IMPORTANCE OF MUSEUM SPECIMENS AS VOUCHERS FOR IDENTIFICATION IN MOLECULAR GENETICS

In recent studies of the phylogenetic relationships in cuckoos and in Old World finches (SORENSEN &

PAYNE 1999, 2001a,b, 2002), both museum skin specimens and tissue samples were used as sources of genetic material. By examining voucher specimens we confirmed or corrected the identification of a number of molecular samples.

These voucher specimens were prepared by the collector and retained by the museum as a document of identification. In most cases, the voucher specimen was located, and examination showed it to be the species as identified by its genetic sequence, and not as in the museum records. In one case the specimen was not located and from the genetic results we suspect the bird was misidentified (case 1): we were able to identify the bird from the collector's measurements. The specimens were correctly identified for most of the 126 tissue samples from six museums, but 8 of these birds (6 %) were misidentified. In cases (1, 2 and 3) the cuckoo tissue was sequenced in another study (JOHNSON et al. 2000) and the incorrect identification (in case (1), a new misidentification as „*Cuculus vagans*“) was published and incorrectly entered into GenBank. Our conclusions about avian systematics and evolution would have been incorrect had we not examined the specimens and field data.

1. A Philippine cuckoo was identified as a hawk-cuckoo *Cuculus (fugax) pectoralis* and the record was published with this identification. The genetic sequence from its tissue was unlike that of other *Cuculus fugax* or *C. pectoralis* that we have sequenced, but it was like oriental cuckoo *C. saturatus*. Neither the two North American museums that supported the fieldwork, nor the Philippine National Museum to which these museums directed us, had a register record of the voucher specimen, said to be an unsexed spirit. The collector provided us with the wing measurement, 181 mm, indicating the bird was *C. saturatus*.

2. A Philippine cuckoo identified as plaintive cuckoo *Cacomantis merulinus* yielded genetic sequence from tissue the same as a Philippine brush cuckoo *Cacomantis variolosus*. Examination of voucher specimens showed that both were *Cacomantis variolosus*, one an adult female and one (the misidentified bird) a juvenile.

3. A juvenile South African cuckoo identified as Klaas cuckoo *Chrysococcyx klaas* had a gene sequence like that of diderik cuckoo *Chrysococcyx caprius* and unlike another *C. klaas*. Examination of the voucher skin showed that the bird was a juvenile *C. caprius*.

4. A Bornean cuckoo was identified as oriental cuckoo *Cuculus saturatus lepidus*. Sequence analysis of the

tissue sample showed it to be like other *Cacomantis variolosus*. The bird was preserved as a study skin for the collection of Sabah Parks. The Research Officer (Zoology) of Sabah Parks photocopied the specimen and its label with the data that matched the data of the collector. The faxed photocopies showed the bird to be a juvenile *Cacomantis variolosus*.

5-9. Five South African ploceids were misidentified to species in a museum that has an active program of collecting genetic samples and voucher specimens. Examination of the voucher specimens gave identifications that were consistent with the genetic sequence information, so the birds had been misidentified by the collectors and the museum.

In another case a voucher specimen verified the museum identification where the identity was questioned in the genetic results.

A number of countries require that specimens remain in the country of origin as a condition of a permit to collect. This requirement can benefit the host country with specimens for scientific development and research, but it can make the specimens unavailable for reference and repeated sampling in molecular systematics (RUEDAS et al. 2000). When specimens are returned to the country of origin, we recommend that the museum take photographs and retain distinctive feathers with a museum registration number. For example, ZMUC saved the entire molted plumage of a unique specimen of a *Laniarius* bush-shrike from Somalia; when the bird was released to the field after a long period in captivity (SMITH et al. 1991), the entire set of feathers was retained as a permanent archival record at the museum.

4. DISCUSSION

A feather itself is both a sample for DNA sequence information and a voucher. Many species can be identified on the basis of a single flight feather or distinctive display feather, and museums can catalogue these with study skins. In our own studies, feathers from avicultural sources were used as vouchers, such as the distinctive barred feathers of pictorella finch *Heteromunia pectoralis*. Feathers removed from a museum specimen can be returned to the museum and reattached to the specimen as in the original skinning process, or labeled and stored in individual envelopes. Blood samples from the same individuals that are used for feather samples for mtDNA sequence might provide nuclear markers to resolve questions of recent hybridization. For market birds, captive birds in avi-

culture, and wild birds caught and sampled for blood or feathers then released in the field, we recommend a photograph for documentation.

Museum collections can provide archives of birds used in genetic study when blood, tissues or feathers lost in the skinning process are preserved separately from the study skin, skeleton and spirit. Continued collecting is recommended (e.g., REMSEN 1995; WINKER 2000): new avian taxa are continually being discovered, our museums undersample the variation of birds of the world, and we can sample the genetics of birds of special systematic and conservation interest. Because not all specimens have been correctly identified in recent genetic studies, we recommend that an active museum systematist be involved in genetics studies to identify the voucher specimens. Finally, we suggest that the importance of museum specimens as primary genetic resources be considered in balancing the views of molecular genetics use as „destructive sampling“ versus „added value“ science.

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