

THE VALUE OF VERTEBRATE TISSUE COLLECTIONS IN APPLIED AND BASIC SCIENCE

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Abstract

Collections of tissues from wild vertebrates (as opposed to cultured tissues) present special problems and opportunities. In general, the public is interested in the ecology and conservation of vertebrates and, thus, may understand the value of vertebrate systematic studies. However, heightened awareness and emphasis on vertebrate natural history create concerns over (1) collecting vertebrate specimens and (2) spending disproportionate amounts of scanty government funds on the study of vertebrates at the expense of other organisms. These feelings in the public and scientific communities currently make it difficult to build vertebrate tissue collections. Such problems are exacerbated by the perceived poor investment potential of vertebrate tissue collections in the private sector; it is thought unlikely that pharmaceutical, agricultural, biochemical, or other investors would reap as much return from vertebrate collections as from some plant or invertebrate collections. However, the potential value of vertebrate collections for basic and applied research is still enormous. The LSU Collection of Genetic Resources, for example, has been a major source of information for research on such disparate topics as bird and mammal conservation, epidemiology of Hantavirus, and forensics, not to mention hundreds of more traditional evolutionary studies.

Introduction

Vertebrate tissue collections consist of frozen or chemically preserved tissues and their genetic extracts, e.g. proteins and DNA, collected from wild or zoo animals. These collections play an extremely important role in genetic research. With the increase in comparative DNA studies brought about by improved DNA technology, the demand for tissues from wild vertebrates has increased dramatically. From 1986-1990, for example, the LSU Museum of Natural Science (LSUMNS) Collection of Genetic Resources dispensed an average of 186 tissue samples per year. From 1991-1995, that average jumped to 463 tissues per year (Table 1). This increased demand stems not only from molecular systematists and population geneticists who traditionally use such collections, but also from an ever-increasing number of ecologists, behaviorists, conservationists, molecular biologists, epidemiologists, toxicologists, agricultural and wildlife researchers, and even law enforcement officials, all of whom need comparative genetic material. It is clear that vertebrate tissue collections are essential for solving problems both of basic and applied science.

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Table 1. Totals of tissues provided for research from the Louisiana State University Genetic Resources Collection.

<i>Year</i>	<i>Number of Grants</i>	<i>Number of Specimens</i>
1985-1989	4	750
1990	19	332
1991	21	459
1992	19	243
1993	33	616
1994	36	328
1995	39	668
1996 through May	21	169
Total	232	3,565

At the same time that demand for vertebrate tissues is increasing, so is the difficulty of building and maintaining vertebrate collections. Vertebrates, as generally large and identifiable creatures, enjoy the benefits of public awareness. To some extent the public even understands the importance of systematic studies of vertebrates, for example, to provide historical and genetic perspective for decisions in conservation. However, heightened public awareness is generally accompanied by concern over the collecting of vertebrate specimens, even of common species or individuals doomed by habitat destruction. Moreover, in the scientific community, there is resistance to spending scanty government funds on the collection of vertebrates when so many other taxa are less well known and more poorly represented in collections. Such problems are exacerbated by the perception in the private sector that vertebrate collections have lower investment potential than other kinds of collections. Pharmaceutical, biochemical, and agricultural companies are more inclined to fund the collection of certain plant and invertebrate groups because of the obvious potential for pay back. Such stories as the discovery and use of disease-resistant wild corn, tree barks with cancer-curing properties, and mollusk slime with anti-fungal properties are increasingly common and understandably enticing to investors (e.g., Wilson 1988). However, although the applicability may be less obvious, vertebrate tissue collections are of paramount value in applied science.

To illustrate the issues facing vertebrate tissue collections, we provide a series of examples of how these collections are used, or may be used, in various areas of basic and applied science. The distinction between basic and applied research is simply a convenience to categorize and simplify the description of research problems. Virtually all of the "applied" uses of vertebrate tissues, even those of epidemiology and forensics, depend heavily on empirical or theoretical observations derived from basic studies in evolution, phylogenetics, population genetics, and other forms of comparative molecular biology.

Demands, Problems, and Responsibilities of Vertebrate Tissue Collections

Demands of Basic Research

Traditionally, vertebrate tissue collections have been used by basic scientists to provide material to study population variation and infer phylogeny. As DNA technology improved during the 1980s, alternative methods for phylogenetic reconstruction and population genetics became widely available, e.g. restriction enzyme analyses, DNA sequencing, and DNA fingerprinting by RAPD's, minisatellites, and microsatellites, and more and more systematists were attracted to molecular approaches. In the late 1980s, the demand on vertebrate tissue collections increased dramatically as this growth in technology combined with theoretical advances in ecology. The most important developments were in the burgeoning field of molecular ecology. One element of this field is "historical ecology." It is based on the recognition by ecologists, behaviorists, and conservationists that understanding life histories and interactions of organisms depends not only upon a description of where they live and what they do-i.e., their ecology and behavior-it requires knowledge of their history-where they come from, who they are related to, and how are they genetically constrained or directed (e.g., Brooks & McLennan, 1991; Harvey & Pagel, 1991). A simple example from our own research is the Australian Tree Martin (*Hirundo nigricans*), which nests in a tree hole and dams the hole with mud. An ecologist acting without phylogenetic information might infer that these swallows dam the hole with mud because mud is the most available substance or it has special insulating or protective properties. An historical ecologist with phylogenetic information would know that all species closely related to the Australian Tree Martin make mud nests; thus, the use of mud may be a genetic requirement or "phylogenetic constraint" of these birds (McKittrick 1993, Winkler & Sheldon, 1993). The field of molecular ecology also includes the use of molecules to study ecological problems that traditionally were studied by simple field observation. The most dramatic example is the use of mini- or microsatellite DNA fingerprinting to establish parentage of individual animals or the structure of family groups (e.g., Quinn et al., 1987).

The adoption of molecular techniques by ecologists has particularly affected vertebrate genetic collections. Because a disproportionately large number of scientists study vertebrates, there are unusually large amounts of data on vertebrate ecology and behavior. To exploit these data, vertebrate scientists are turning to comparative genetics to build phylogenies or to examine population and family structure. As a result they are combing vertebrate tissue collections for comparative material. Although vertebrate tissue collections enjoy the increased attention, they have difficulty meeting the increased demand.

Demands of Applied Science

Applied scientists have placed less pressure on the holdings of vertebrate tissue collections than basic scientists, but their needs reveal the *archival* importance of

tissue collections. Typically, applied scientists require reference material to compare to DNAs or proteins they already possess or to identify patterns within or among populations. For example, forensic scientists need standards to calibrate measurements or identify samples to species; wildlife or agriculture officials often need multiple examples from populations to establish population parameters on game or pest species; epidemiologists often need multiple samples from a single population that have been collected over a long period of time, or widely dispersed samples of a species collected over a short period of time, to establish the range, development, or pathway of an infectious agent; molecular biologists need comparative material to establish such phenomena as horizontal gene transfer or homology of genetic material to develop probes and primers; conservationists and wildlife officials need comparative material to establish the genetic variation of populations and historical genetic records when deciding which stock is appropriate for repopulating areas where individuals have been extirpated or reduced (e.g., Lande & Barrowclough, 1987). Vertebrate genetic collections regularly provide materials for these kinds of studies as well as other applied uses.

A common theme that unites essentially all applied scientific uses of vertebrate tissue collections is the *establishment of a historical genetic pattern*. That is, there is a pervasive need to know how populations vary over time and space. The epidemiologist and toxicologist must identify when and where an infection or toxin entered a population; the conservationist must know how and why a population changed in genetic make up; a pest-controller must determine from where his pest derived. Such questions are most readily answered if collections possess multiple samples of species over a wide area and long period of time. Thus, vertebrate collections are most valuable if they obtain specimens of many taxa continuously over broad areas. Short-term locally restricted collections may be useful for specific problems or studies, but in general they will not provide an adequate perspective of historical genetic change in time and space. Vertebrate tissue collections are able to provide the required information if they contain substantial holdings as a result of general collecting.

Problems and Pressures

Unfortunately, in most circumstances vertebrate tissue collections cannot meet the demands of the growing number of systematists, ecologists, and applied scientists. Most vertebrate tissue holdings are semi-formalized, private, research collections that have been accumulated for specific projects and are not large or organized enough to meet modern demand, either locally or globally. Only about 10 collections in the world have wild vertebrate tissue holdings in excess of 10,000 specimens, and many of these are highly specialized or localized (Dessauer et al., 1996). In addition, all tissue collections are struggling to develop loan, administration, ownership, and other policies, and are faced with severe bureaucratic problems concerning collecting and handling permits. The latter are particularly pressing.

Perhaps no problems concern vertebrate tissue collections more than opposition to collecting by protectionists and the difficulty of obtaining collecting permits im-

posed by arbitrary government permitting processes. The theoretical and practical issues of vertebrate collecting have been thoroughly reviewed (e.g., Finley, 1988; Winker et al., 1991; Remsen, 1995). It is clear from our perspective that government agencies should base their conservation policies on the protection of habitats and populations, not individual animals, and that the logic and strategy of this habitat approach be understood and adopted by permit-granting agencies. Without more enlightened and reasonable policies on collecting, the future and usefulness of vertebrate tissue collections are severely threatened.

In addition to the difficulty of collecting specimens are the bureaucratic problems associated with importing, housing, and distributing vertebrate tissue samples. These are quite remarkable, as the following examples will show. In order to import bird tissue and associated voucher specimens from Costa Rica to the United States, in addition to Costa Rican collecting and export permits (and CITES export permit if necessary), the following permits are required: USDA Tissue Import Permit; a USFWS Migratory Bird Import Permit, if applicable; an OMA CITES I Permit for species on CITES I Appendix; and a USFWS Non-designated Port Exception, in case the cheapest flight happened to come in to Houston, instead of Miami or New Orleans. In some cases, this Non-designated Port Exception is no longer required. See 50CFR (13,14) Final Rule, U.S. Federal Register, June 21, 1996. USFWS and USDA have to be notified in advance of the shipment's arrival, and an original plus two copies of USFWS Form 3-177 have to be turned in, along with copies of all of the other permits when declaring the specimens at Customs. Moreover, your institution has to be approved as an "establishment" by the USDA to house the incoming material legally.

Sending tissue grants to a researcher at another institution invokes a different assortment of rules and regulations. If, for example, you need to send an aliquot of tissue of a North American passerine to a researcher outside of the US, the following permits and clearances are required: MBI Permit, for most species requiring 30 days notice; copy of appropriate USFWS/State collecting permits; and clearance of the shipment by a USFWS inspector at a designated port prior to shipment (again Form 3-177 and two copies); and, if the species is listed in one of the CITES appendices, a CITES Institutional Permit is required by both institutions. If imported bird tissues are transported to another U.S. facility, that facility has to obtain a USDA Transport Permit if already inspected and approved by the USDA; if not, the receiving facility has to be inspected and approved by the USDA. The USDA takes a minimum of 2 weeks to issue a Transport Permit and a minimum of 4 weeks for institutional inspection. Cooperation between and within government agencies and with scientific institutions during the permit process varies greatly from region to region.

Responsibilities

In addition to these stringent requirements, we emphasize the growing responsibilities of tissue collections. These include the obvious, such as good record keeping and organization, fast delivery of samples to researchers, and raising money to maintain freezers and pay for curation. But there are also less appreciated responsibilities.

First, tissue collections have the responsibility to archive not only tissues but voucher specimens (Winker et al., 1996). A recent development among comparative vertebrate geneticists is to avoid collecting whole specimens by simply taking a genetic sample (blood or biopsy) and releasing the animal alive (e.g., Smith et al., 1992). However, instances of misidentified or mixed samples in molecular analyses are common, and without voucher specimens it is often difficult to sort out the source of the problem. Moreover, vouchers are often required to supplement genetic data with morphological data, and this need may not be recognized until after the molecular comparisons are completed. This is particularly true in studies of geographic variation, toxicology, and epidemiology, which may require morphological verification of molecular patterns.

Second, tissue collections must accept the responsibility for tracking and managing extracts derived from tissues. These extracts - including DNA and proteins - are the products of destructive sampling of tissues. Whether they derive from frozen tissues, excised parts of traditional museum specimens, including extinct species, or directly from wild organisms, such extracts have almost as much research value as their source tissue. Although some extracts find their way into museum collections, most do not. Molecular researchers are often unaware that some collections are willing and capable of storing tissue extracts. Other researchers may believe that it is impractical to deposit molecular extracts in museum collections (Whitfield & Cameron, 1994). As a result, tissue extracts are often relegated to the back of a freezer, eventually to be lost or thrown away as the researcher moves on either physically or intellectually. Even if small private collections of extracts are cared for, their value is diminished because their contents are unknown and thus unavailable to the majority of the research community. Because tissue extracts are unique and often nonrenewable, it is imperative that they receive maximal usage (Hafner, 1994). To this end, they should be deposited in major repositories, that are widely recognized as genetic research resources. By virtue of their equipment and organization, major repositories can accession and care for such extracts over the long term.

Examples of Uses of Vertebrate Tissues and Extracts from the LSUMNS Collection

Basic Research

The LSUMNS Collection of Genetic Resources has distributed more than 3,500 tissues in the last 10 years to researchers in more than 10 countries studying phylogeny and population genetics of vertebrates. In Table 2, we provide examples of some of those loans. In compiling the list, we have attempted to select a broad range of projects to illustrate the variety of basic research that relies on vertebrate tissue collections. Because the LSU Collection is probably the largest of its kind in the world, this list should reflect current use of such a collection fairly reliably, with perhaps a bias towards birds, reptiles, and amphibians as a reflection of our strength in those areas.

Applied Research

In Table 2, we have also included loans of tissues for applied purposes marked with asterisks. Because these loans reflect an under-appreciated contribution of vertebrate tissue collections, we discuss some of them below.

Epidemiology and Hantavirus - In May and June 1992, there was an outbreak of Hantavirus in the Four Corners area of New Mexico, Colorado, Arizona, and Utah that killed 24 persons (Nichol et al., 1993). The virus has erupted with similar deadly effects in other locations as well, including Kansas, Louisiana, and New York. Hantavirus infects small rodents and is transmitted in the airborne dust of their dry feces (Baker, 1994; Marshall, 1993). The 1992 outbreak is attributed to the deer mouse, *Peromyscus maniculatus*, but other species carry Hantavirus as well (e.g., the marsh rice rat, *Oryzomys palustris*, in Louisiana). By studying the presence of this virus in these animals, we are in a position to develop a remarkable understanding of its epidemiology. Fortunately, deer mice and some of the other carriers are unusually well represented in vertebrate tissue collections, e.g. LSU, University of New Mexico, Texas A&M, and Texas Tech, because they are easy to trap and commonly used in teaching and population biology research. As a result, these collections can provide tissues to determine (1) if the virus has been present in various populations since the inception of tissue collections, (2) which populations it has historically infected, (3) whether it has recently mutated to a virulent form causing the infection of humans, and (4) how infectious the virus may be under different circumstances, as indicated by collectors' notes that accompany specimens (Baker, 1994; Marshall, 1993). There is perhaps no clearer example of the scientific reward that comes from collecting large numbers of specimens both at single sites over a long period of time and across broad areas.

Genetic Variation and the Spotted Owl - The spotted owl (*Strix occidentalis*) has been a lightning rod for public concern over logging of old growth forest in the American northwest. The issues of spotted owl conservation are complicated by political factors, but among them are questions about the genetic variability of the Pacific populations (*S. o. caurina* and *S. o. occidentalis*), and genetic differentiation between the Pacific and allopatric southwestern populations (*S. o. lucida*) (e.g., Barrowclough & Gutierrez, 1990). These questions bear on the health and potential longevity of the Pacific populations and whether nonthreatened and threatened populations are conspecific. LSUMNS does not have spotted owl tissues, but has been able to provide an unusually large sample of tissues from a single population of a close congener, the barred owl (*S. varia*). This large sample has permitted the researchers who are examining spotted owl population genetics to determine how much variation to expect in a healthy, independent, population of large *Strix* owls (G. Barrowclough, pers. comm.).

Hybridization of the Dusky Seaside Sparrow, and the search for its closest relative - Five of the six last remaining dusky seaside sparrows (*Ammodramus maritimus nigrescens*) were brought into captivity. The captives (all males) were mated with the Gulf Coast subspecies in an effort to preserve the remaining dusky genes. The Gulf Coast population was chosen based on its proximity and similar morphology

to dusky seaside sparrows. The proposed plan was to backcross the successive generations of sparrows to arrive at a new "dusky seaside sparrow." This plan failed, and subsequent genetic analysis based largely on samples in tissue collections showed that it was ill-conceived from the outset. Dusks are most similar genetically to Atlantic, not Gulf Coast, members of the species (Awise & Nelson, 1989). This was a classic study, because it indicated the importance of phylogenetics to conservation management.

Python Perpetrator - In May of 1993, a man from Harahan, Louisiana, was purportedly killed by his 5m, 90kg, pet reticulated python (*Python reticulatus*). In investigating the death, the Louisiana State Laboratory in the Public Safety Services Department obtained from the LSU Collection some reticulated python plasma and antisera to use in their blood typing.

Yellow-crowned Night-Heron, a Cajun Delicacy - Forty tissues of various heron and ibis species have been sent by the LSUMNS Collection to the USFWS Forensic Laboratory in Eugene, Oregon. Researchers at the lab are developing biochemical markers and working in conjunction with USFWS Law Enforcement officers in Louisiana to identify contraband heron meat. Yellow-crowned night-heron (*Nyctanassa violacea*), as well as white ibis (*Eudocimus albus*), are still considered delicacies in the ethnic Acadian portion of the State. Although protected by the Migratory Bird Treaty, these species continue to be shot, often in large numbers. These hunts sometimes eradicate entire local rookeries. The meat is sold illegally at markets and served at ethnic restaurants to select clientele. It is difficult to catch poachers in the act, and the development of markers would allow confiscated meat to be identified to species and persons in possession of contraband meat to be prosecuted.

Summary

We emphasize two important features of these examples. The applied uses of vertebrate genetic tissues relies on relatively large samples taken over long time or wide space. They are also unpredictable. Thus, to be effective, vertebrate tissue collections must cover as many species and contain as many specimens as possible.

Table 2. Examples of the variety of research supported by tissue grants from the LSU-MNS Genetic Resources Collection. Five requests/year are selected for years 1991-1995.

Year	Researcher	Institution	Details of gift and project goals
1991	M. Heath	Environmental Medicine, U.S. Navy Medical Research Lab, Bethesda, Maryland	Two Chinese pangolin (<i>Manis peniadactyla</i>) samples to be used to develop a DNA fingerprinting probe
1991	P. Sereno	Dept. Organismal Biology & Anatomy, University of Chicago	4 ostrich and rhea samples for study of basal bird relationships
1991	L. Maxson	Penn State University, University Park, Pennsylvania	15 antisera samples of Caribbean <i>Anolis</i> for phylogenetic analysis
1991	R. Ricklefs	Dept. Biology, University of Pennsylvania	86 avian samples for a historical biogeographic study of Lesser Antillean passerines
1991*	G. Barrowclough	American Museum of Natural History, New York	14 samples of barred owl (<i>Strix varia</i>) to study population variation in a common congener of the endangered spotted owl (<i>Strix occidentalis</i>)
1992	S. Edwards	Dept. Pathology, University Florida	20 jay samples for a study of the evolution of major histocompatibility complex (MHC) genes
1992	A. de Querioz	Sec. Ecology & Systematics Cornell University, Ithaca	6 squamate samples for study to determine phylogenetic position of snakes
1992	R. Furness	Zoology Dept., Glasgow University, Glasgow, Scotland	41 blood samples of skua for phylogenetic analysis of Stercorariidae
1992	J. Wilkinson	Biological Laboratory, Kyoto University, Kyoto, Japan	36 frog tissues for frog phylogeny
1992	G. Veron	Museum National D'Histoire Naturelle, Paris, France	6 samples of carnivores for study of evolutionary systematics of Malagasy Carnivora

1993*	C. Baker	Louisiana State Lab, Public Safety Services, Baton Rouge	Python antiserum for use in a forensic study to ascertain if cause of death was the result of pet python
1993	T. Quinn	Dept. Biological Sciences, University of Denver	21 duck samples for NSF-supported research into relative rate of sex vs. autosomal chromosome evolution
1993	D. Weishampel	The Johns Hopkins University School of Medicine	10 hummingbirds for mtDNA-based phylogeny of hummingbirds
1993	T. Dehm	Sam Houston State University, Huntsville, Texas	16 <i>Sigmodon hispidus</i> samples for population genetics study
1993	A. Knight	Louisiana State University Medical Center, New Orleans	Elephant shrew, mole, shrew, and bat samples for an investigation of chromosomal transposable elements
1994	B. Tobalski	Dept. of Physiology, University of Montana, Missoula	6 woodpecker samples for histological analysis of physiological correlates of flight behavior of woodpeckers
1994*	L. Eggert	Center for Reproduction of Endangered Species, San Diego	Wren tissues to compare in phylogenetic approach to management of endangered San Diego cactus wren (<i>Campylorhynchus brunneicapillus sandiegense</i>)
1994	H. Ellegren	Swedish University of Agricultural Sciences, Sweden	66 swallow samples for study of microsatellite DNA polymorphism
1994*	S. Donnellan	Dept. of Genetics, South Australian Museum	4 least tern (<i>Sterna antillarum</i>) to compare with little tern (<i>S. albifrons</i>) in taxonomic study with emphasis on protection and management in Australia and Japan
1994*	R. Fleisher	U.S. National Zoo, Washington D.C.	12 <i>Rallus</i> samples for a US-FWS-funded population study in anticipation of clapper rail (<i>R. longirostris</i>) translocation

1995*	B. Hjelle	University of New Mexico, School of Medicine	95 <i>Oryzomys palustris</i> for search for the true host of hantavirus strain "Bayou virus" after fatal case in Monroe, LA
1995*	W. Ferguson	National Fish & Wildlife Service Forensic Lab, Ashland, Oregon	39 heron and ibis samples to use in identification of genetic markers to identify contraband heron meat
1995*	T. Glen	Molecular Systematics Lab, Smithsonian Institution, Washington, DC	32 Whooping Crane (<i>Grus americana</i>) blood samples
1995	I. Lovette	Smithsonian Tropical Research Institute, Panama City, Panama	103 warbler samples for analy- sis of phylogeny and evolution of Neotropical warblers
1995	S. Dunham	Dept. of Biology, University of Nevada	10 <i>Otus</i> samples for project to ascertain phylogenetic relation- ship of flammulated owl (<i>O. flammeolus</i>) to New vs. Old World <i>Otus</i>

Asterisk (*) following date indicates applied science projects.

References

- Avise, J. C. & W. S. Nelson. 1989. Molecular genetic relationships of the extinct Dusky Seaside Sparrow. *Science* 243: 646-648.
- Baker, R. J. 1994. Some thoughts on conservation, biodiversity, museums, molecular characters, systematics, and basic research. *J. Mamm.* 75:277-287.
- Barrowclough, G. F., & R. J. Gutierrez. 1990. Genetic variation and differentiation in the Spotted Owl (*Strix occidentalis*). *Auk* 107:737-744.
- Brooks, D. R., & D. A. McLennan. 1991. *Phylogeny, Ecology, and Behavior*. Univ. Chicago Press, Chicago.
- Dessauer, H. C., C. J. Cole, & M. S. Hafner. 1996. Collection and Storage of Tissues. pp. 29-47 In: D. M. Hillis, C. Moritz, & B. Mable (eds.), *Molecular Systematics*. Sinauer, Sunderland, Massachusetts.
- Finley, R. B. Jr. 1988. Guidelines for the management of scientific collecting permits. *Wildl. Soc. Bull.* 16:75-79.

- Harvey, P. H., & M. D. Pagel. 1991. *The Comparative Method in Evolutionary Biology*. Oxford University Press, New York.
- Hafner, M. S. 1994. Reply: Molecular extracts from museum specimens can—and should—be saved. *Mol. Phyl. Evol.* 3:268-278.
- Lande, R., & G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management, pp. 87-123 In: M. E. Soulé (ed.). *Viable Populations for Conservation*. Cambridge University Press, Cambridge.
- Marshall, E. 1993. Hantavirus outbreak yields to PCR. *Science* 262:832-836.
- McKittrick, M. C. 1993. Phylogenetic constraint in evolutionary theory: Has it an explanatory power. *Ann. Rev. Ecol. Syst.* 24:587-619.
- Nichol, S. T., C. F. Spiropoulou, S. Morzunov, P. E. Rollin, T. G. Ksiazek, H. Feldmann, A. Sanchez, J. Childs, S. Zaki, & C. J. Peters. 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 262:914-917.
- Quinn, T. W., J. S. Quinn, F. Cooke, & B. N. White. 1987. DNA marker analysis detects multiple maternity and paternity in single broods of the lesser snow goose. *Nature* 326:392-394.
- Remsen, J. V. Jr. 1995. The importance of continued collecting of bird specimens to ornithology and bird conservation. *Bird Conservation International* 5:145-180.
- Smith, E. G. F., P.; Arctander, J. Fjeldsä, & O. G. Amir. 1991. A new species of shrike (Laniidae: *Laniarius*) from Somalia, verified by DNA sequence data from the only known individual. *Ibis* 133: 227-235.
- Wilson, E. O. 1988. *Biodiversity*. National Academy Press, Washington, D.C.
- Whitfield, J. B., & S. A. Cameron. 1994. Museum policies concerning specimen loans for molecular systematic research. *Mol. Phyl. Evol.* 3:268-278.
- Winker, K., B. A. Fall, J. T. Klicka, D. F. Parmelee & H. B. Tordoff. 1991. The importance of avian collections and the need for continued collecting. *Loon* 63:238-246.
- Winker, K. B., M. J. Braun, & G. R. Graves. 1996. Voucher specimens and quality control in avian molecular studies. *Ibis* 138:345-346.
- Winkler, D. W., & F. H. Sheldon. 1993. Evolution of nest construction in swallows (Hirundinidae): A molecular phylogenetic perspective. *Proc. Natl. Acad. Sci. USA* 90:5705-5707.