Chapter 15

Guidelines for the Use of Herbarium Materials in Molecular Research

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Abstract.—Destructive sampling of herbarium specimens for morphological and anatomical studies has been with us for a long time, and most herbaria have implemented at least an informal policy to deal with such requests. With the advent of molecular systematics, sampling has taken on an added dimension and has forced herbaria to deal with some basic questions regarding this new use of specimens. Discussion of these issues among morphological and molecular systematists and the curatorial staff at the Harvard University Herbaria has resulted in the development of a general policy on sampling; in addition, it has raised questions regarding the storage of DNAs and associated molecular data that must be addressed by the botanical community as a whole. Although it is not yet possible to formulate a precise policy regarding the return and storage of DNAs, we hope that by providing a set of guidelines at this stage we will be better able to monitor activities in this area and work toward a community-wide agreement on an optimal solution.

From the earliest days of natural history collections, curators have dealt with the necessity of balancing conservation of those collections with maximum use for the advancement of science and society. Recognizing that these specimens provide a permanent record of the existence of individual organisms, curators and researchers

using those collections have been mindful of maintaining the integrity of each specimen. In instances where removal or dissection of a portion of the material was essential, permission was usually granted by the curator on the merit of the individual request; such sampling has been an element of many plant morphological studies over the years. Within the last decade, sampling for morphological and anatomical work has been supplemented by sampling for molecular studies, where technical advances have made obtaining DNA samples from specimens not only feasible but practical. Here we discuss the ways in which the Harvard University Herbaria (A, AMES, ECON, FH, GH, and NEBC) have been dealing with the issues raised by this new use of herbarium specimens.

With the advent of molecular systematics, destructive sampling of herbarium specimens takes on an added dimension. Over the last decade, the development and refinement of laboratory techniques for the extraction of DNA have proceeded rapidly, enabling researchers to use smaller and smaller amounts of plant material in order to obtain sufficient DNA for analysis, especially polymerase chain reaction-based approaches (Loockerman and Jansen, 1996). It was not unexpected, therefore, that researchers began to turn increasingly to herbaria as a source of plant tissue; herbarium specimens are readily accessible, represent a wide variety of taxa, and essentially eliminate the necessity of collecting and identifying additional material.

Yet, while requests for destructive sampling for DNA studies are increasing, many herbaria have not given serious consideration to policies and procedures for handling such requests. Recent discussions of general collections policies by Cato (1993) and Cato and Williams (1993) underscore the need for institutions to address these issues before they become a problem. In the case of Harvard University Herbaria, the establishment of three new laboratories for molecular studies in the building, and the relatively active loans program already in place in the herbarium, were the prime incentives for establishing a policy statement to deal with such requests for removal of material from herbarium specimens.

The logical first step for us at Harvard Herbaria was to initiate a general discussion among morphological and molecular systematists and herbarium curatorial staff. These discussions raised several questions. How likely would it be for researchers to use herbarium specimens for molecular work? How much material was needed for a typical isolation procedure? Were herbaria running the risk of being perceived as simply warehouses for researchers interested in a ready supply of DNA? If sampling of specimens for DNA studies was allowed, could it be adequately monitored, and could the community insure that specimens of relatively rare taxa — which might be in high demand for broad-based phylogenetic studies — be protected? In short, the immediate concerns were whether DNA sampling was something that we wanted to support, and, if so, what policies should we adopt for dealing with such requests?

The first question was answered in our initial round of discussions. The consensus was that sampling for molecular studies was indeed an important new use of herbarium specimens, and that making those specimens available to the scientific community would represent a significant new contribution. At the same time, it was obvious that guidelines were necessary to insure that the collections were used properly. It was critical to establish how best to balance this single use — destructive by its very nature — with preservation of the material for future needs. Subsequent discussions focused on these issues, and our resulting policy statement (Appendix I) provides the basis for dealing with requests for destructive sampling of herbarium specimens, including molecular studies. Specifically, we require that researchers return to Harvard Herbaria some product of their work, be it SEM photographs of pollen, slides of wood, or a portion of the extracted DNA. For molecular studies, we require that a protocol be sent to us in advance, that the results be reported to us in writing, and, as with any loan, that the specimen be annotated with the identification accepted by the user as well as notation of what material was removed for study. Unless additional packeted plant tissue was collected specifically for a given kind of study (e.g., air-dried leaf material to be used expressly for DNA extractions), we normally only allow removal of material from a sheet once for a given category of study, such as pollen for SEM studies. In cases of large or complicated requests, we encourage researchers to seek their own funds to visit Harvard Herbaria and select specimens themselves, with removal of material and/or permission for removal given under the supervision and approval of the

appropriate staff. Also, we encourage researchers to search available living collections first before any sampling of herbarium specimens is initiated. Several requests for material sent to Harvard Herbaria, especially for Asian taxa, have been at least partially filled with material from the living collections at the Arnold Arboretum of Harvard University. That resource is renewable, the material is usually ample, and our experience has been that arboreta and botanical gardens are quite willing to make their collections available.

STORAGE OF DNA SAMPLES

DNA samples and results are only now beginning to be sent back from researchers. A loan to the University of Arizona is illustrative: a loan of seven specimens of the tribe Galegeae (Fabaceae) was sent for study and sampling (Wojciechowski and Sanderson, University of Arizona, pers. comm.) following receipt of a formal loan request from the Herbarium, University of Arizona (ARIZ), a signed authorization form, and an extraction protocol from the researchers. The returned loan was accompanied by six DNA samples, precipitated and air-dried, and a detailed written summary of the methods and results, both positive and negative. The DNA samples are being stored at –80°C in our molecular laboratories, along with extractions from other plant groups (some dried and some in buffer) made by staff and students of the Herbaria.

The return of a portion of extracted DNA samples to Harvard Herbaria is perhaps the part of our policy that is most controversial and experimental. Recent discussions of this issue by Whitfield and Cameron (1994a, 1994b) and by Hafner (1994) underscore important considerations about the role of museums as repositories for specimens and their derived data. We agree with the recommendations of all the authors that molecular extracts from specimens be deposited in museums equipped with facilities for long-term storage. In the case of the Harvard Herbaria, the request for a portion of the extracted DNA to be returned to us is being made on a trial basis, with results to be evaluated periodically. Depending upon the long-term value of the samples, the frequency of requests for their use, and the efficacy of long-term storage, we may modify our policy to either drop the requirement altogether, make the parameters more specific, or consolidate samples to a more centralized storage institution. In summary, we view our current policy statement as a work-in-progress, subject to change as the parameters become better defined.

The fact that we are developing another collection — not unlike the herbarium itself, or the wood or slide collections — requires us to consider the following questions:

1) If we are in the business of storing DNA samples, what can we do to foster their use? Is it realistic to envision a coordinated effort among herbaria, or some subset of repository herbaria, in which DNA is stored, the information databased, and the DNA made accessible to other researchers on request?
2) Is it really possible to effectively store DNA extracted from herbarium specimens — or DNA extracted from live organisms — for long periods of time, in such a way that the integrity of a sample is maintained, and additional molecular data can be obtained?

With the return of DNA to Harvard Herbaria as a condition of our policy, combined with a significant level of in-house activity, we have begun to establish our own repository for DNA samples; other herbaria with associated molecular laboratories are in a similar position. A simple database, developed by the second author, links each DNA sample to the corresponding herbarium specimen (Fig. 1). Further developments of this database might include its availability over the Internet via Harvard Herbaria's World Wide Web site (http://www.herbaria.harvard.edu), and coordination with Harvard Herbaria's existing Type Specimen Database, which tracks specimens via a unique bar code number. However, the question remains whether we as individual institutions and herbaria should continue to pursue individual and yet parallel paths with respect to DNA storage. An alternative would be to designate some group of repositories within the botanical community which could serve as clearinghouses for sample requests (Adams, 1994; Hafner, 1994; Whitfield and Cameron, 1994a). The latter course seems preferable to us, and is also practical since many institutions may not have the resources to store DNA samples. In addition, access to information can be streamlined if there are a few central repositories; this would also make it easier to develop database standards and links.

FIGURE 1. Entry in DNA sample database.

SAMPLE

Sample nr	1
Loc_freezer	Donoghue Lab
Loc_box	1
Sample type	Raw DNA
Amount	100 uL
Extract. data	Regular CTAB (2x) with PVP (1%)
Source	Herbarium specimen
Responsible	Torsten Eriksson
Received	94-05-10
GenBank nr	U41381
Literature	Eriksson, T., and M. J. Donoghue. 1995. Phylogenetic analyses of *Sambucus* and *Adoxa* (Adoxaceae) based on nuclear ribosomal ITS sequences and morphology. Amer. J. Bot. Suppl. 82: 128 (abstract 367).
Note	

Taxon

Family	Adoxaceae
Genus	Sambucus
sp.	australasica (Lindl.) Fritsch
ssp.	
var.	
form	

Voucher specimen

Collectors	Schodde
nr	5172
date	13 Dec. 1966
Herbarium	A
Acc. nr.	
Country	Australia
Det. by	

LINKS BETWEEN DATA AND SPECIMEN

In their discussion of the issue of return of DNA to the lending institution and subsequent storage, Whitfield and Cameron (1994a) cited "little coordination of the storage of molecular systematic data with more traditional taxonomic sources of information." This problem of data coordination is a serious one which has plagued conceptually linked yet physically isolated collections for years. Harvard's own wood collection is an example: in some instances, the link between the wood sample housed in the Bailey-Wetmore Laboratory and its corresponding voucher specimen in the herbarium has been lost. However, advances in computer technology and widespread use of the Internet and World Wide Web will allow links between molecular data and biological specimens to be developed so that the data can be made readily available to the community at large. Blake et al. (1994) summarized a recent meeting on the interoperability of biological databases, in which representatives of seventeen separate databases met to review technical and semantic issues pertinent to integrating data from various molecular sequence, citation, specimen, nomenclature, and phylogenetic relational databases, and to formulate mechanisms for queries among these databases. Although problems exist both from the technological (i.e., multi-database relational queries) and systematic (i.e., standardization of taxonomic names/classifications) perspectives, these efforts will advance the cause of ready access to biological data of all sorts. In the meantime, databasing efforts such as TreeBASE (http://phylogeny.harvard.edu/treebase/) for storage of phylogenetic trees and data matrices (Sanderson et al., 1994) and the Sequence, Sources, Taxa (SST) database (http://www.tigr.org/tdb/sst/sst.html) for linking gene sequences to voucher specimen data (Blake and Bult, 1996) can serve as repositories for information that is now accumulating at an astonishing rate. As Thomas (1994) noted, databases such as SST make a critical link between the derived molecular data and their underlying source, pointing "back to the final arbiter, a curated specimen in a collection."

LONG-TERM STORAGE OF DNA EXTRACTED FROM HERBARIUM SPECIMENS

Even if the botanical community were soon to reach a consensus on DNA repositories and database links, a fundamental question remains: have we still such a long way to go in perfecting the technical aspects of DNA extraction and storage that a community-wide effort is not yet appropriate? DNA from herbarium material is often degraded and in low concentrations, and it can degrade further over a short period of time (Jansen et al., this volume). Jansen has indicated that some DNAs from herbarium material will not amplify easily after freezer storage for one year, making re-isolation necessary. Some of these problems may be circumvented by the use of laboratory procedures yielding ultra-clean extracts, such as ultracentrifugation with cesium chloride. In any event, before centralized repositories are established, basic questions about the stability of current extractions must be answered. Laboratories involved in molecular extractions, including our own, need to initiate long-term experiments in which samples kept under a variety of controlled conditions are subsequently tested for usability. Until these issues are clearly resolved, our intention is to continue to solicit a portion of the extracted DNA from researchers as a condition of our loans; however, this policy will be reviewed often, and we may eventually concentrate instead on storage of *information* relating to a given specimen and its associated molecular data.

RESPONSIBILITIES OF THE MOLECULAR AND MUSEUM COMMUNITIES

To date, our experience at Harvard Herbaria with the practice of sampling for molecular studies has been a positive one. We have endorsed the use of herbarium specimens for such studies within a prescribed set of guidelines that were developed as mentioned above with the full cooperation of molecular and morphological systematists and the curatorial staff of the Herbaria. At the same time, we have strengthened our commitment to the concept that individual herbarium specimens are a non-renewable resource.

A variety of issues related to sampling still need to be addressed before we will be sure of the value of long-term storage of DNA samples. We have addressed the question of the physical integrity of DNA samples, but the problem of possible contamination owing to carelessness in the laboratory also needs more attention (Mueller, this volume).

All systematists have a responsibility to insure that voucher specimens exist for their work, and they must take every opportunity to renew the resource whenever they are in the field. Efforts to store silica-gel or air-dried material to supplement vouchered collections are currently underway at several herbaria today (Chase and Hills, 1991; Miller, this volume). Field collectors should make use of these techniques to collect leaf tissue as part of their regular collecting routine. Today's systematists, regardless of the nature of their research, need to continue to train their students in plant collecting procedures in order to insure well-prepared voucher specimens for studies of all sorts and adequate material for the future.

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