CHAPTER 18

Destructive Sampling and Information Management in Molecular Systematic Research: An Entomological Perspective

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Abstract.—The small size and large numbers of insect specimens handled by entomological collections present special vouchering and information management problems. There is, at the same time, an increasing trend toward integration of the results of molecular and more traditional morphology-based systematic studies. As a result, entomological museums, journals, and systematists need to develop consistent policies for the deposition of voucher specimens and for the management and databasing of the information derived from these specimens. A number of challenges in the designation of voucher specimens from molecular research are discussed, and several recommendations are made to meet these challenges. The advantages of specimen-based data management are discussed; additional recommendations are made to facilitate the integration of molecular systematic data into general specimen-based taxonomic databases.

INTRODUCTION

Molecular systematic research on insects, as on other groups of organisms, is relatively recent. Only within the last two decades have systematists applied molecular data to phylogenetic analyses, although population genetic studies using allozymes, and physi-

ological and developmental investigations using protein and DNA sequences date further back. The appearance and refinement of polymerase chain reaction (PCR) technology (Mullis and Faloona, 1987; Kessing et al., 1989; White et al., 1989; Innis et al., 1990; Simon et al., 1991) have revolutionized the efficiency and affordability of obtaining DNA sequences from insects, including both live and preserved specimens. A number of introductory guides and protocols are now available for PCR and DNA sequencing of insects (Cameron et al., 1992; Brower and DeSalle, 1994; Hoy, 1994; Simon et al., 1994). Consequently, the use of preserved museum specimens for molecular studies is increasing and will likely continue to do so because they provide access to a wider range of taxa and localities than might normally be available to a researcher and may also reveal short-term evolutionary or distributional changes.

In spite of this, most systematic collections have not developed standard protocols for the use of their specimens in molecular research. The lack of such protocols has become a significant issue because, unlike traditional morphological sampling techniques, the process of converting tissues to DNA or protein extracts may result in partial or full destruction of the specimen. Thus there is a need for new policies governing the use of specimens from systematic collections in molecular systematic research.

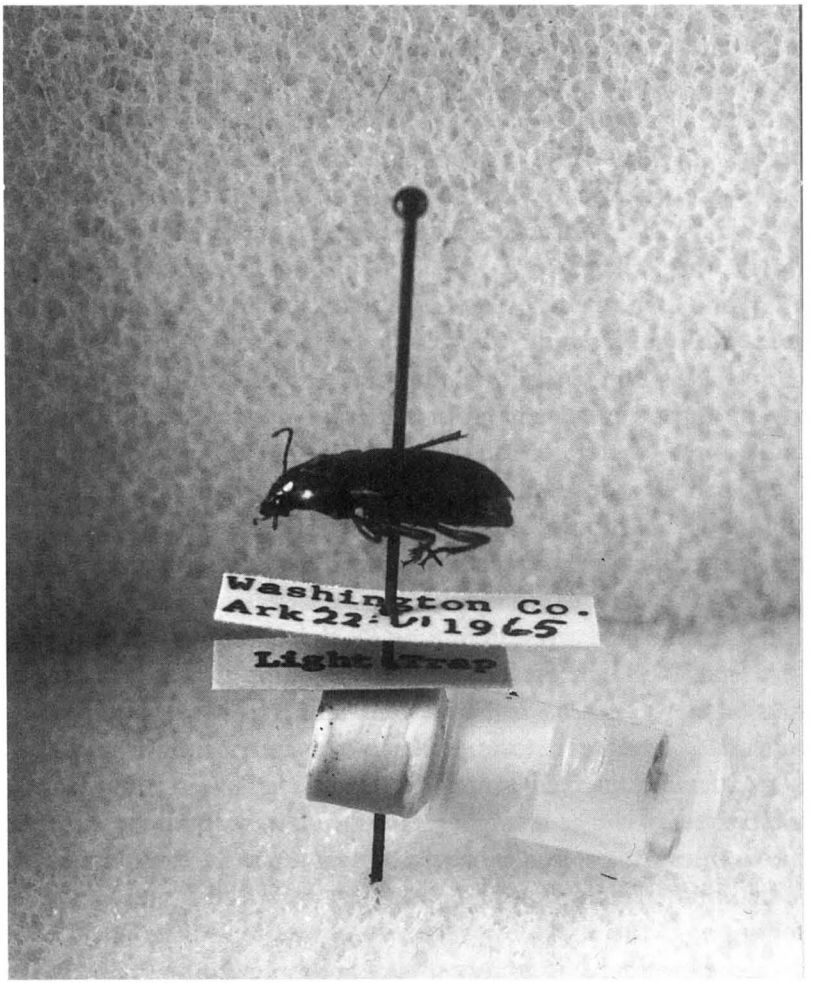
This paper reviews a number of factors affecting the use of specimens for molecular research from an entomological perspective, including destructive sampling, preservation and storage techniques, and the long-term viability of DNA extracts. It then turns to the need to retain fragments and collection data of destroyed specimens, and to link them with the data resulting from molecular research. The recommendations offered should be applicable to other collections of animals, plants, and fungi.

THE ISSUE OF DESTRUCTIVE SAMPLING

The concept of destructive sampling is not new. Specimens from insect collections have long been dissected to reveal genitalia, mouthpart, and internal characters, or for making slide-mounts of wings or other body parts to facilitate their study. In a few cases,

specimens preserved in fixatives are loaned for destructive comparative studies of internal anatomy. Usually, the specimen is returned with its dissected portions either contained in a small vial pinned with the specimen (Fig. 1) or mounted on a microscope slide. Museums have generally required preparator code numbers and allied notebooks to associate the specimen with the extracted information and body parts, although no uniform format has been developed to manage these data.

FIGURE 1. Pinned specimen of a carabid beetle, with preserved genitalic dissection associated with it using a genitalia vial.



Specimens used for DNA or protein analyses differ from most of the applications mentioned above: portions are not simply removed from the original specimens, they may be pulverized for extraction of the relevant molecules. Except for analysis of the extracted molecules, the removed portions of the specimens are no longer useful. The extracted molecules are also ultimately consumed in the course of the research.

In theory, one can amplify target DNA from a small fragment of a modern or fossil specimen, preserving the remainder for other uses. In reality, a number of factors can affect the DNA yield of a specimen, reducing it to the point that an insect specimen may be fully consumed. Some specimen-preservation methods can either lower DNA extraction yields (Post et al., 1993; Dillon et al., 1996; S. Cho, UC, Berkeley, pers. comm.) or directly or indirectly affect PCR efficiency. DNA is best preserved in freshly-caught or freshly-frozen (–80°C) specimens. Refrigeration enhances the preservation of tissues for DNA and morphological analyses alike (Masner, 1994), thus the next best source of DNA is specimens which were killed in the field in 95–100% ethanol and kept at 4°C. Dried, pinned specimens generally yield low quality, degraded DNA, although those killed in ethanol appear to yield more DNA (Dillon et al., 1996) than those that have simply died in killing bottles. Formalin-preservation destroys the DNA entirely and is therefore not recommended. The method of specimen preservation will become less important as molecular techniques are modified to become more sensitive, and with more taxon-specific oligonucleotides for PCR. Eventually increasingly smaller fragments of nearly all types of insect specimens may be useful for molecular research using PCR.

It may not be necessary in all cases for fully curated insect specimens to be used for molecular research. Most entomology collections have bulk stores of unsorted material from trap collections that is preserved in 70-95% ethanol. If these samples are transferred to 100% ethanol and refrigerated, they will be valuable storehouses for molecular research, at least as valuable as determined pinned collections. At present, however, this practice is uncommon.

Some molecular techniques, such as those used for allozyme or

restriction site analysis, require fresh or frozen specimens rather than alcohol-preserved material. Currently only a few museums (Hafner, 1994) maintain collections of frozen specimens or tissues at ultra-cold temperatures. However, the procurement of such specially preserved insect specimens (frozen and/or ethanol-preserved) by museums will surely increase in the future, as their value becomes clear. They will likely be maintained in specialized facilities, separate from the principal, pinned voucher collections, similar to those described by Engstrom et al. (this volume) for mammals, and will be available on loan for molecular research.

When museum specimens loaned for molecular research must be destroyed, in whole or in part, the researcher should justify the use of the material for this purpose. Moreover, the researcher must demonstrate experience in the appropriate molecular techniques and methods of analysis. For example, researchers might be required to submit a brief research proposal, including pilot results if available. This would allow the museum to make informed decisions, weighing the scientific benefits of the research against the long-term cost to the collection and its future users.

Museums should be considering each of these issues as they begin to formulate policies that will take them into the twenty-first century, as molecular techniques becomes fully integrated into systematic research.

DNA EXTRACTS AS VOUCHERS

There has been recent debate over the proposal that systematic collections request the return of DNA extraction aliquots to serve as vouchers for material used for molecular work (Hafner, 1994; Thomas, 1994; Whitfield and Cameron, 1994a, 1994b). Several museums currently require the researcher to submit aliquots of DNA from specimens borrowed for molecular investigations (e.g., Harvard University Herbaria, The Natural History Museum, London). Several other institutions are prepared to receive aliquots of frozen DNA and tissue samples for long-term storage (Dessauer and Hafner, 1984; Dessauer et al., 1990; Hafner, 1994). Hillis and Moritz (1996) report on long-term tissue storage. Despite this, there is no general consensus among molecular systematists

and museum curators supporting the mandatory requirement of returning DNA aliquots to the lending institution. This is not to imply that extracts should be discarded after use, but rather that the issue of permanent storage may have to be evaluated on a case-by-case basis, depending on the long-term viability of the extract and on the ability of the museum to properly curate it. In this era of dwindling museum budgets, it is unrealistic to expect many, let alone all, institutions to commit to long-term, high quality storage of frozen material.

In time, we will be better equipped to assess the long-term prospects of vouchering DNA. For the time being, when assessing the long-term usefulness of stored DNA extracts, museum curators must consider several factors. First, a variety of DNA extraction protocols has been used with insects (e.g., Simon et al., 1991; Cameron et al., 1992; Cameron, 1993). These differ widely with respect to purification procedures and the stability of the DNA extract because there is often a trade-off between ease and efficiency of extraction on the one hand and long-term stability on the other. Second, the long-term viability of DNA extracts under ultra-cold conditions is not yet fully known. However, preservation of ethanol-precipitated and/or dried DNA extracts may be superior to resuspended products.

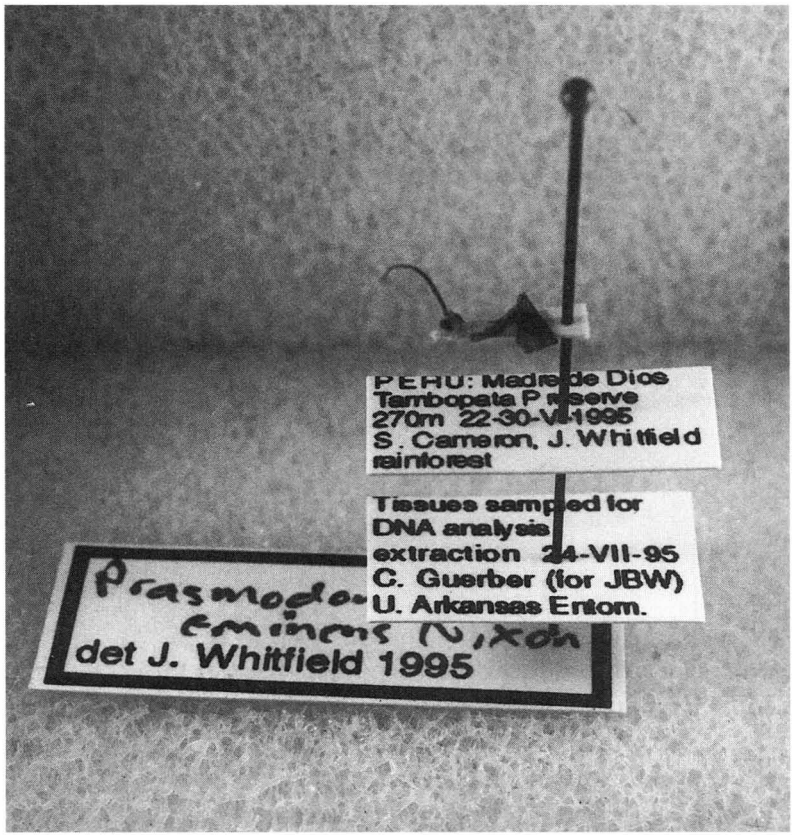
Until then, it is reasonable to expect molecular systematists to save DNA extracts whenever possible, report the storage location of specimen extracts to the lending institution, and reference the location of relevant extracts in scientific publications. An ultimately more permanent and taxonomically useful form of vouchering may be preserving and documenting those parts of the museum specimens not sacrificed for molecular extraction.

REMNANTS OF SPECIMENS AS VOUCHERS

Most specimens of plants or vertebrates are large relative to the small sample of tissue required for DNA extraction. In contrast, insect specimens are small relative to the size of the tissue sample needed for DNA extraction.

Small insects are customarily ground *in toto*, though often wings and other appendages are removed before grinding. These

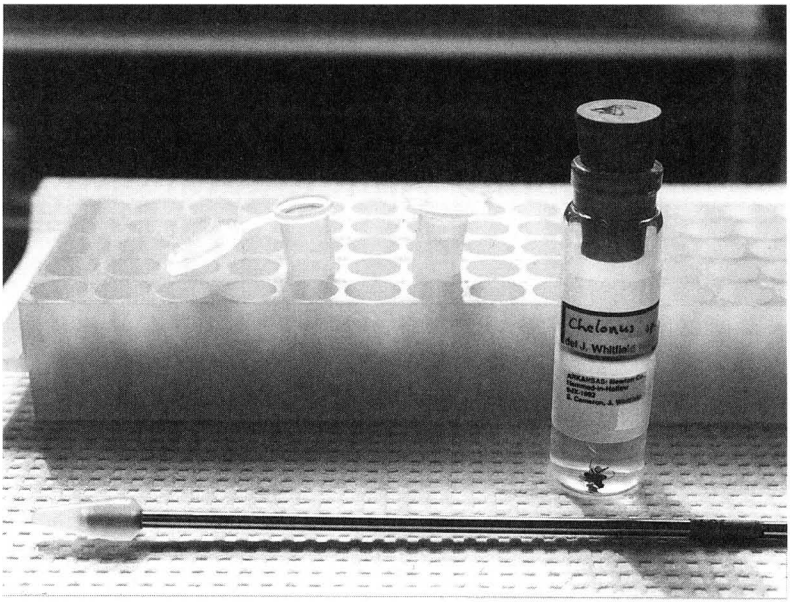
FIGURE 2. Mounted remnants from a braconid wasp specimen used in a molecular phylogenetic study. A second label is attached below the collection data label, outlining the use of the specimen in molecular research.



removed parts should be saved and retained with the original data labels (Figs. 2 and 3). However, small remains may not always suffice to verify the identification of a specimen at a later time. Therefore, it is best to save an additional specimen believed to be conspecific or from the same population, if available, which could in future be compared with the specimen parts.

With larger insects, a smaller portion of the specimen can be taken, such as the thorax or portions of it, or only one side of a bilaterally symmetrical animal may be used. The thorax contains a large amount of muscle tissue, rich in mitochondria and low in lipid

FIGURE 3. Small insect specimens are often ground for extraction in an Eppendorf tube using a small Teflon pestle. For good results, nearly an entire specimen the size of this braconid wasp may need to be used.

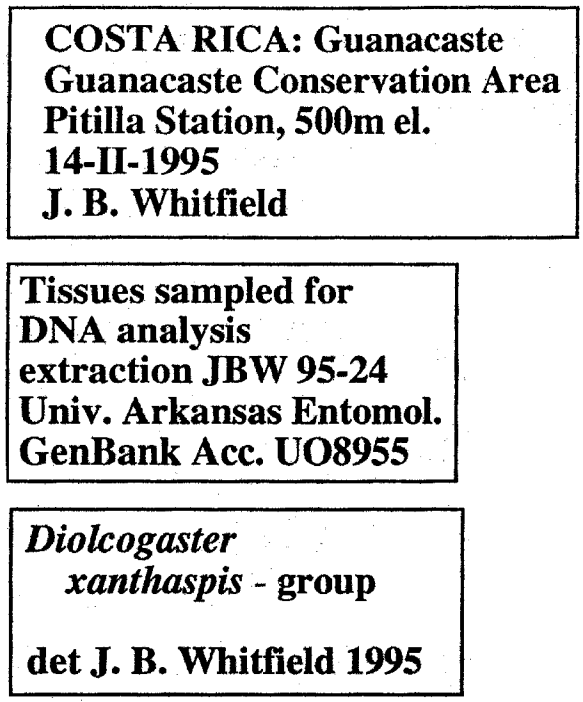


content, which produces relatively high yields of DNA. Furthermore, it contains fewer endosymbionts which can complicate phylogenetic analyses. Remnant portions of larger insects are probably sufficient as voucher specimens, as long as care is taken to preserve portions of the animal used in identification.

To date, many published molecular phylogenetic investigations have not documented remnant voucher material. Those authors that have used material borrowed from museums have generally been more conscientious. The fault is probably two fold. On the one hand, some molecular systematists are not familiar with vouchering protocols. On the other hand, museum collections have often not been adequately prepared to accept this material. To correct this, museums must develop the organizational framework for accepting remnant voucher specimens from all molecular systematic studies. The remnant voucher specimen, accompanied by its original label (collection locality and other information), should be incorporated into the main collection. This will allow

systematists and ecologists to integrate information from the sampled specimen into a larger assessment of morphological and geographical relationships of an entire taxon, and to assess the need for further DNA sampling. An additional label (Fig. 4) should be kept on or with the DNA sample. It should include the essential information required to associate it with the remnant specimen voucher, including the researcher's name and address and the accession numbers for relevant DNA sequences stored in GenBank, EMBL or other databases.

FIGURE 4. Example of data labels associated with a voucher specimen from molecular phylogenetic research. The second label contains information related to its sampling, extraction, and resulting data. Note: the GenBank accession number is not yet assigned for this actual specimen; the number is hypothetical but of the form used by GenBank.



The scientific value of linking molecular data with relevant specimen vouchers stored in systematic collections that serve as permanent repositories cannot be overemphasized.

SPECIMEN-BASED DATA RETRIEVAL

Systematic collections, which already have data storage and retrieval systems in place, are a natural repository for vouchers (intact specimens or fragments thereof) from molecular systematic studies. Many collections are developing linked, computerized databases (Blake et al., 1994) and it would be valuable to also link data from molecular systematic research with taxonomic and phylogenetic databases (Thomas, 1994).

Many collections are moving toward the use of standardized bar code technology for tracking specimens and for linking specimens to other data. Bar codes greatly simplify the organization and retrieval of specimen-based data in computer databases. The entomological-museum community (through the Entomology Collections Network) has voted to use a standard, high density bar code, Code 49, for all entomological specimens. This is the first step to creating a single, compatible database system. The ultimate goal is to be able to electronically link all data of systematic interest to specific taxa.

Thus far I have presented the view that systematic collections should be fully involved in the storage and management of all information linked to specimens. Not all curators may agree with this outlook, either philosophically (Hafner, 1994) or for economic reasons, as tight budgets may limit a museum's role to that of archival storage. I argue that museums can and should manage specimen information and, likewise, play a major role in the development of taxon databases. In fact, the increasingly important role of museums in information management can be a strong justification for increases in funding.

CHANGES IN PUBLICATION REQUIREMENTS

Editors of journals and other publications can play an important role in specimen-based information management for molecu-

lar research. Many journals (e.g., *Proceedings of the National Academy of Sciences, Molecular Phylogenetics and Evolution*) require authors to submit sequence data to GenBank, EMBL, or an equivalent database and to report accession numbers for all taxa. All journals should make this a requirement.

Rarely are authors of molecular investigations required to give collection data for specimens, or state the location of voucher specimens. Yet such information is required of authors publishing taxonomic revisions based on comparative morphology, ecology, and behavior. This lack of attention to standard systematic practices may have become routine during the early days of molecular systematic research, when many analyses were concerned with questions of higher level relationships (e.g., kingdoms, phyla), or focused on well-known taxa (e.g., anthropoids). As it becomes increasingly common for molecular phylogenetic studies to address questions at lower taxonomic levels, and to include taxa of less certain status, we must apply the same standards for publishing as those that exist for more traditional taxonomic studies (i.e., collection-data documentation and voucher deposition). It is imperative that results of current molecular phylogenetic research be testable in the future. Hence, editors of journals that publish the results of molecular systematic research should require a high standard of specimen documentation.

SUMMARY OF RECOMMENDATIONS

Several guides have been developed to aid institutions in developing policies and guidelines for the use and management of collections (Cato, 1993; Cato and Williams, 1993; Hoagland, 1994). These guides deal broadly with the problem of destructive sampling. The following recommendations, together with those made in other chapters in this volume, offer additional guidance for the formulation of policies governing the use of specimens for molecular systematic research.

1. Each collection should develop criteria for allowing destructive sampling of their specimens.
2. Requesting researchers should be required to: a) submit a short proposal describing the project, presenting pilot results if available,

and estimating how much material will be needed for molecular extraction; b) submit a follow-up report on results and publications based on material, sequence databank accession numbers, and location of remaining extracts (if these are not also returned); and c) acknowledge the loaning and/or voucher institutions in any resulting publications.

3. The collection should have adequate storage and retrieval mechanisms for the above information.
4. Collections may require that an aliquot of each extract be returned either to them, or to another institution with facilities for long-term storage.
5. The standard voucher requirements applied to comparative morphological systematic research should also be applied to molecular systematic research. Voucher or remnant voucher specimens for each individual studied should be deposited in a permanent collection. This will facilitate future identification of and research on a particular taxon.
6. All journals publishing the results of molecular systematic research should require that each submission include complete collection data for specimens used, location of voucher specimens, and sequence database accession numbers (if relevant).

To ensure that the above recommendations can be achieved, communication between the systematic collections community and the molecular systematics community should be encouraged and enhanced.

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