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## Insect collections and DNA analyses: how to manage collections?

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Numerous papers have been published recently regarding deoxyribonucleic acid (DNA) studies of museum insect specimens. Despite the great enthusiasm for such analyses, there continues to be a potential conflict between specimen maintenance and their use for experimental procedures, since DNA extraction methods are sample destructive. This has created a strong limitation for studies on museum specimens, making it impossible to work on rare or irreplaceable species. However, new methods for improving insect preservation have been recently published, together with new procedures for performing less destructive DNA extractions that combine specimen maintenance and DNA extraction.

**Keywords:** DNA analysis; entomological museum collections; museum collection management; DNA extraction

### Introduction

Recent innovations in molecular biology techniques have increased the importance of deoxyribonucleic acid (DNA – the carrier of genetic information) studies in evolutionary and conservation biology (Carter, Vogler, and Vane-Wright 1997; Dean and Ballard 2001; Fukatsu 1999; Hillis, Moritz, and Mable 1996; Quicke, Belshaw, and Lopez-Vaamonde 1999). At the same time, the availability of 'easy to apply' molecular techniques has led to an increased interest in museum collections, since they are extremely useful for studying extinct species at the molecular level and for performing biomolecular analyses on specimens previously studied at a morphological level only (Carter, Vogler, and Vane-Wright 1997; Dean and Ballard 2001; Fukatsu 1999; Hillis, Moritz, and Mable 1996; Quicke, Belshaw, and Lopez-Vaamonde 1999; Vogler and Pearson 1996).The beginning of several DNA barcoding projects has also increased the interest in museum collections, since they offer a huge amount of vouchered samples that can give rise to worldwide barcoding campaigns without any new field collection (Hebert et al. 2002; Sovalainen et al. 2005).

In the wake of this enthusiasm for recovering DNA from museum specimens (e.g., Cooper 1994; Houde and Braun 1988; Payne and Sorenson 2003), museum curators and conservators realized that there was a potential conflict between maintaining specimens and using them for experimental procedures (Graves and Braun 1992; Payne and Sorenson 2003). The fact that extraction methods are

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destructive for the samples comes up against the need to maintain irreplaceable specimens. The damage to the collection, therefore, has to be balanced against the 'value' that molecular analyses adds to the studied specimens (Payne and Sorenson 2003).

The amount of tissue needed for molecular assays is becoming smaller and smaller, thanks to different genomic DNA amplification procedures, but a minimum amount of sample (whose quantity can vary in different taxa) is still required. However, taking very small portions of tissue to preserve specimen integrity can be a false economy, since it can yield no genetics data. Moreover, the extracted DNA could also be damaged and not useful for biomolecular studies (Graves and Braun 1992; Payne and Sorenson 2003). Museum curators and conservators should, therefore, determine whether specimens that can be sampled will have significant value, or if the costs in terms of damage or specimen loss are greater than the expected scientific interest.

**Effects of archival maintenance on insect preservation**

DNA is a relatively chemically stable molecule, but it does not preserve well under all storage conditions (Lindahl 1993; Quicke, Belshaw, and Lopez-Vaamonde 1999). The main problems that occur if stored specimens are used for molecular studies are DNA shearing and DNA inter-strand cross-linking (Dessauer, Cole, and Hafner 1996; Golenberg, Bickel, and Weihs 1996; Hofreiter et al. 2001; Lindahl 1993). Shearing is the degradation of DNA into small fragments and it is caused by several factors, including exposure to radiation (mainly UV), temperature, pH and sample age (Dean and Ballard 2001; Dessauer, Cole, and Hafner 1990; Lindahl 1993; Mandrioli, Borsatti, and Mola 2006). These factors make insects that are not immersed in fixative solutions extremely fragile and frequently not useful for molecular analyses.

DNA fragmentation could have effects on both DNA extraction and amplification, as short fragments precipitate less efficiently than long ones, leading to reduced yields after DNA extraction. Moreover, DNA degradation could make unsuccessful the polymerase chain reaction (PCR) amplification of long DNA fragments (Dessauer, Cole, and Hafner 1996; Lindahl 1993). Inter-strand cross-linking is due to various chemical reactions, such as alkylation (the addition of an alkyl group to nucleotides), that inhibit denaturation and consequently DNA amplification during PCR (Kornberg 1980). The occurrence of inter-strand cross-linking blocks the progression of the DNA polymerase on the DNA template, affecting PCR efficiency (Dean and Ballard 2001).

At present, ultra-cold freezing at $-80^{\circ}$C and liquid nitrogen are the two most effective methods for specimen storage, since both gave results that are comparable to those obtained using fresh samples after DNA extraction and PCR amplification (Mandrioli, Borsatti, and Mola 2006). However, specimens stored in this manner are not always available and, in particular, it could be difficult to use these methods during field surveys or for storing large amount of samples.

Materials that have been specifically collected in the field for genetic analysis furnish easy to obtain DNA of high quality (Payne and Sorenson 2003). In order to carry through with this high-quality process, proper tools for collecting, and infrastructure for storage, need to be in place. The growing popularity of DNA

barcoding and molecular taxonomy in general emphasizes the increased need for such practices and facilities.

In view of such difficulties, several alternative and practical methods have been applied to preserve insect samples for DNA studies (Dean and Ballard 2001; Dessauer, Cole, and Hafner 1996; Dillon, Austin, and Bartowsky 1996; Fukatsu 1999; Mandrioli, Borsatti, and Mola 2006; Post, Flook, and Millest 1993; Quicke, Belshaw, and Lopez-Vaamonde 1999). In particular, it seems extremely important to remove water as quickly as possible from insect tissues in order to avoid DNA degradation. Some coleopterists (specialists in beetles) and dipterists (specialists in flies) rapidly desiccate specimens by placing them in an airtight container with silica gel (Quicke, Belshaw, and Lopez-Vaamonde 1999). Larger insects can be quickly and efficiently desiccated only by employing critical point drying that leads to well-preserved specimens (Post, Flook, and Millest 1993; Quicke, Belshaw, and Lopez-Vaamonde 1999).

The best fixative solutions for insects differ depending on cuticle composition. A survey of various techniques in the literature failed to identify a unique, widely applicable and cost-effective archival medium (Table 1). The lack of precise details about insect storage methods increases the possibility of an inappropriate storage and indicates the need for further investigations. For instance, cold 70 and 100% ethanol solutions are reported (Quicke, Belshaw, and Lopez-Vaamonde 1999) as highly effective in Hymenoptera (bees, wasps and ants), whereas both were scarcely effective in Lepidoptera (butterflies and moths) as reported by Mandrioli, Borsatti, and Mola (2006), and in Coleoptera (Reiss, Schwert, and Ashworth 1995). This difference could be due to the slower ethanol entrance into lepidopteran and coleopteran tissues, as reported in large-sized hymenopteran species. This problem has been solved in Hymenoptera by making small holes in the thorax and abdomen with a micro pin that allow alcohol to infiltrate them (Quicke, Belshaw, and Lopez-Vaamonde 1999). This suggests that DNA degrades less if it is quickly saturated in

Table 1. Literature survey for the most effective preservation methods in insects for molecular studies.

| Order | Best storage medium | Maximum storage time tested[a] | Reference |
|---|---|---|---|
| Ryncota: Homoptera | Acetone at room temperature | Three years | Fukatsu (1999) |
| Blattodea | Acetone at room temperature | Two years | Fukatsu (1999) |
| Isoptera | Acetone at room temperature | Five years | Fukatsu (1999) |
| Lepidoptera | Acetone at room temperature | Two years | Mandrioli, Borsatti, and Mola (2006) |
| Hymenoptera | 70 or 100% ethanol at low temperature (at least 4°C) | 35 years | Quicke, Belshaw, and Lopez-Vaamonde (1999) |
| Coleoptera | Silica-gel desiccation | 40 years | Gilbert et al. (2007) |
| Diptera | Silica-gel desiccation | Three months | Post, Flook, and Millest (1993) |

[a]The reported times refer to the maximum sample ages that gave successful results in DNA extraction and PCR amplification.

alcohol (Quicke, Belshaw, and Lopez-Vaamonde 1999). The use of absolute ethanol (Flournoy, Adams, and Pandy 1996) is preferred to alcoholic solutions (e.g., 95% ethanol, 1% methanol and 4% water), or to an alcoholic drink such as vodka (Oakenfull 1994), since DNA is better preserved and these solutions contain some contaminants that can affect the PCR process (Quicke, Belshaw, and Lopez-Vaamonde 1999).

A quick and effective insect preservation has been reported using acetone solutions in Coleoptera, Lepidoptera, Isoptera and Homoptera (Fukatsu 1999; Mandrioli, Borsatti, and Mola 2006). In all cases, acetone was effective at room temperature making this solution cheap and easy to use in the archival maintenance of insect collections. Acetone-preserved insects have also been analyzed at a histological level showing that it allows both DNA and morphology preservation (Fukatsu 1999). On the contrary, poor results have been generally reported with other fixatives, such as methanol, Carnoy's solution, chloroform, 2-propanol and EDTA salt (Dean and Ballard 2001; Dessauer, Cole, and Hafner 1996; Fukatsu 1999; Mandrioli, Borsatti, and Mola 2006; Post, Flook, and Millest 1993).

### Storage time and storage environments

Degradation of DNA in insect specimens occurs over time, depending on the preservation technique. However, it has been shown that with preservatives mixed with water (e.g., 70% ethanol), and with those that enter tissues slowly (e.g., Carnoy's solution and methanol), the DNA extraction results are worse than when no preservative was used. Indeed, the wrong fixatives cause DNA shearing within a few days, making it impossible to use specimens for biomolecular analysis. On the contrary, dried, pinned insects, not exposed to fixatives, can be used for DNA analyses, although the percentage of successful specimens after both DNA extraction and PCR is quite variable (Quicke, Belshaw, and Lopez-Vaamonde 1999). The main problem with dried, pinned insects is that it is impossible to know beforehand if specimens will provide molecular data, making the evaluation of the balance between sample damage and the added value difficult. The choice of the right fixative solution can assure museum curators about the possibility of having results after sampling.

In all cases, the time of storage had a significant effect on the length of the sequence that can be amplified by PCR, so that an increase in the sample age corresponds to a reduction in the amplicons length (Dean and Ballard 2001). However, even after years, it is possible to amplify relatively short fragments (about 600–700 base pairs) that allow, for example, the use of collected insects for DNA barcoding projects (Mandrioli, Borsatti, and Mola 2006).

### Destructive vs. semi-destructive sample management

DNA extractions are destructive for the analyzed specimens, and sampling may compromise the whole body of the insect. This irreversible damage to specimens prompts museum curators to see molecular techniques as unattractive for rare and important specimens (such as type specimens and rare or extinct insects) (Gilbert et al. 2007; Payne and Sorenson 2003). To overcome these limitations, semi-destructive methods have been developed to extract DNA with minimal, visible,

external morphological damage to the specimens (Gilbert et al. 2007; Skevington, Kehlmaier, and Stahls 2007; Starks and Peters 2002).

Semi-destructive methods can use portions of insects to extract a sufficient amount of DNA for molecular assays, such as a three to five millimeters (mm) long portion of one or two legs (e.g., Hebert et al. 2004; Ivanova, DeWaard, and Hebert 2006; Skevington, Kehlmaier, and Stahls 2007; Starks and Peters 2002). This approach has been applied to Diptera, Hymenoptera and Lepidoptera quite frequently, and DNA can be extracted using the legs of one side, with the adult retained in the museum as a voucher specimen (e.g., Hebert et al. 2004; Ivanova, DeWaard, and Hebert 2006; Skevington, Kehlmaier, and Stahls 2007; Starks and Peters 2002). Legs are particularly useful in Lepidoptera, since pinned butterflies and moths are maintained in the entomological cabinets with the legs below the body, so that it is not immediately evident that specimens have been dissected.

A very interesting chemical extraction method has been recently established for Coleoptera that allows DNA sampling from dried museum specimens, without conferring external morphological damage (Gilbert et al. 2007). In particular, whole beetles are immersed in an extraction buffer that digests the soft internal tissue, leaving the exoskeleton unaltered. Studied insects can be successively saved in the entomological cabinets for further studies (Gilbert et al. 2007). A similar chemical DNA extraction has been successfully applied to different terrestrial arthropods and, in particular, to specimens belonging to Acarina (mites and ticks), Araneae (spiders), Coleoptera, Diptera, and Hymenoptera, chosen to represent the ranges in size, overall sclerotization and delicacy of key morphological characters (Rowley et al. 2007).

The availability of successful and easy to apply semi-destructive DNA sampling methods is very important because it allows molecular analyses and the retention of a morphological voucher for the sequences. Indeed, it is important to assure that each DNA sequence is linked to the original specimen voucher (Winker, Braun, and Graves 1996). If actual specimens are not available, it can be useful to take some photographs of the distinctive features of the samples (Payne and Sorenson 2003). In this regard, it should be noted that molecular studies can be very useful to define sample misidentification that can be successively confirmed by museum systematists (Payne and Sorenson 2003). The occurrence of a voucher for each sequence is so important that it should represent a sine qua non requirement for all published sequence-based results (Ruedas et al. 2000). The importance of the voucher is related to the repeatability of the obtained sequences and to access to the original samples for re-examination, in order to solve questions about species identification, phylogeny and evolution (Ruedas et al. 2000).

The availability of semi-destructive methods allows the combining of molecular data with specific morphological traits of the studied specimens, coupling molecular databases with morphological ones (Rowley et al. 2007). Maintenance of such databases will be necessary and important. For example, if an insect has been used for DNA extraction it could be labelled as ‘used for DNA extraction’. Similarly, if legs have been taken from an insect, the label could state ‘legs away for DNA extraction’.

## Conclusions

In the last decades, an increasing number of molecular studies used insects from museum collections (Suarez and Tsutusi 2004). However, the availability of specimens has been limited by the destructive nature of the DNA extraction procedures that create a conflict between DNA studies and the archival maintenance of the collections. However, recently published studies suggest cheap and applicable methods for improving insect preservation for molecular studies. At the same time, new and less destructive methods of DNA extraction have been suggested which combine specimen maintenance and DNA extraction. These innovations add priority to the need for widely applicable curatorial and research guidelines for molecular assays on museum collection.

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