11 Obtaining, Storing and Archiving Specimens and Tissue Samples for Use in Molecular Studies

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Contents

Collection, storage and archiving of specimens and tissue samples are prerequisites for the successful acquisition of molecular data for any systematics or population genetics study. Field collections represent the most obvious source for tissues, but there are many ways in which tissues can be obtained without ever going into the field. Stock centers and culture collections, commercial supply companies, seed and spore banks, botanical gardens and zoological parks provide an array of live organisms, fresh tissues, cultures or cell lines, while vast quantities of frozen, desiccated or preserved tissues are stored in the collections of frozen tissue facilities, natural history museums and herbaria, among others. This chapter reviews the more important practical aspects of the selection of appropriate tissues for protein or nucleotide extraction, the preservation and temporary storage of freshly collected tissues in the field and the acquisition of specimens and tissue samples from alternative sources. Practical guidelines for the transportation, long-term storage and archiving of tissue samples are presented, emphasising the importance of identification, documentation and voucher specimens to scientifically validate the results of a study. Finally, legal and ethical issues concerning the collection, transportation and storage of specimens and tissue samples, the loan and deposition of samples in collections and the publication of results obtained from their analysis, are addressed from international and U.S. perspectives.

1 Introduction

The obvious first step in any systematics or population genetics study is to focus on a group of organisms or a level of interest. We assume that these issues are self-explanatory and the reader is referred to the second section of this book for examples of the application of molecular techniques to a wide range of questions and a summary of the problems that can arise during the course of such studies. Equally important in the initial stages of the study are sampling strategy, collection, storage, vouchering and archiving. These last two points are especially critical since no standard protocol currently exists for the disposition of tissue or DNA vouchers to scientifically validate the results of a study. Fortunately, this situation is changing as various museums and research universities establish biorepositories for the long-term storage of genetic resources.

There are several ways in which tissues can be obtained for analysis, each with its own peculiarities, nuances and requirements. The purpose of this chapter is not to give an exhaustive account of collecting techniques, but rather to focus on the more important aspects of preservation and storage for successful isolation of the molecules of interest. Other publications

have reviewed the plethora of field collection methods that exist for organisms as diverse as plants, fungi, vertebrates and invertebrates and the reader is advised to consult literature relevant to the taxa of interest for specific details about collecting these organisms. In this chapter we focus on five aspects of sampling and storage: 1) selection of appropriate tissues for protein or nucleotide extraction 2) storage of freshly collected tissues in the field 3) obtaining tissues from other sources, e.g., museum collections, stock centers and commercial supply companies 4) transportation, long-term storage and archiving of tissue samples and voucher specimens and 5) legal and ethical issues involved in the collection, transportation and storage of tissues.

2 Selection of Appropriate Tissues for Molecular Studies

Before embarking on a field trip to collect fresh samples or requesting samples from an official repository, commercial company or informal source, the researcher must decide what tissues are required for the study. Depending on the size of the organism or the kind of material available, DNA or proteins can be extracted from tissue samples or from a homogenate prepared from the whole organism or "clonal" collections of organisms, such as isofemale lines [1]. Tissue selection for molecular isolation depends on the organism to be studied and on the type of molecular work to be conducted. The aim is to use parts of the organism that are relatively free of compounds potentially damaging to the protein or nucleic acid of interest or that may interfere with cloning, PCR, sequencing or restriction digestion. Using specific tissues has the added benefit of reducing the risk of contamination with host or parasite tissues, gut flora or recently ingested prey. For example, DNA extraction from parasitic *Cuscuta* required using only internode tissue to prevent DNA contamination from its host plant species [2].

Young, actively growing leaves or shoots are the best tissues for DNA extraction from plants, but seeds, roots, flowers, stems, pollen, spores and gametophytes have all been used successfully [2–4]. Brown [5] reported successful amplification of DNA from the charred remains of wheat seeds.

Among animals, successful amplifications have been achieved from bone, feathers, scales, hair, muscle, skin, whole blood (liquid and dried), serum, stomach contents and feces, among others. The following tissues, listed in order of desirability, are recommended for DNA extraction from vertebrates: brain, testes or ovary, liver, kidney, heart and skeletal muscle [1]. The nucleated erythrocytes of nonmammalian vertebrates provide a convenient source of DNA because they lack tough connective tissue [6], while milk provides an alternative source of DNA from mammals [7]. Toe clips are a common source of DNA for lizards and anurans [8] and scute

notches provide a nondestructive alternative for chelonians [9]. Fecal and hair samples similarly offer noninvasive options for genetic sampling of mammals, especially endangered species [10–12].

Embryos are an exceptional source of insect mtDNA [1]. However, gonad or muscle tissues are generally suitable for both nuclear DNA and mtDNA isolation from arthropods and must be dissected out, avoiding the exoskeletal material if possible, since the presence of lipids and subcutaneous pigments may inhibit PCR. If specimens can be induced to spawn (for taxon-specific methods, see Strathman [13]), gametes may be obtained without the need for dissection. However, PCR may be inhibited if eggs or sperm are used, because of DNA viscosity [14]. Eggs are preferable to sperm for mtDNA isolation.

If gamete or muscle tissues are difficult to obtain, body tissues *sans* gut provide an alternative. For example, Koch et al. [15] extracted DNA from the heads of blackflies (*Simulium vittatum*) to avoid contamination by parasitic nematodes. Animals too small to be dissected should be starved for at least two days and homogenised whole. Even when all the above procedures are impractical, it is still possible to obtain good results with whole organisms, especially if PCR primers or DNA probes are sufficiently taxon-specific to circumvent possible contamination. For example, Dorn et al. [16] successfully preserved and amplified the DNA of *Trypanosoma cruzi* from human blood samples.

Irrespective of which tissues are selected, they should be used, frozen or preserved immediately, before they begin to degrade. Fresh tissue is preferable if purified mtDNA is required, as freezing may break mitochondrial membranes, reducing yield at the step in a protocol where mitochondria are pelleted. However, if genomic DNA or total cellular DNA with a mtDNA fraction is desired, frozen tissue can provide high yield and quality.

High molecular weight DNA is required in applications such as cloning and RFLPs. In handling tissues intended for such studies, care should be exercised not to allow warming of the tissue after initial storage. Similarly, if protein analysis is intended, care should be taken to maintain specimens at temperatures that will prevent protein denaturation.

Although high molecular weight DNA is preferred for PCR, degraded DNAs will often amplify if the target fragment is small enough and the primers specific enough. DNA intended for PCR is routinely used from both desiccated and ethanol-preserved museum specimens, which may yield fairly degraded DNA. As progress continues with protocols developed for ancient samples, such issues may become *passé*. Interested readers should consult the *Ancient DNA Newsletter*, *DNA Amplifications* (the newsletter of Perkin-Elmer Inc., Wellesley, MA) and the journal *BioTechniques* (Eaton Publishing Co., Natick, MA) for updates of such technological

advances. The website of the Molecular Biology Techniques Forum (Appendix 1) also provides a useful platform for questions.

3 Storage of Freshly Collected Specimens and Tissue Samples in the Field

Field collections represent the most important source for tissues, because the origin of the material (including habitat or provenance data) is fully documented. Additional advantages include the ability to obtain rare or poorly collected species (which can seldom be obtained from other sources), to collect within and among populations, and to reduce the time prior to DNA or isozyme extraction. Disadvantages include the time and expense associated with expeditions to remote areas, together with problems of transportation and permits (addressed below). Nevertheless, many unusual and important molecular systematics and population genetics studies, particularly those addressing biogeographic issues and rare, range-restricted or endangered species, cannot be conducted without field collections.

The method of tissue storage or preservation in the field is determined primarily by the nature of the study – the hierarchical level (population, species, higher taxon) focused on, the molecules (DNA, RNA or isozymes) to be examined, what protocol will be effective for extraction of the molecules, and how pure or intact they must be. Ultimately, the methods of choice will be constrained by logistical factors, most importantly the duration of the field trip, the facilities available and the transportation options. Readers interested in pursuing molecular studies requiring visitation and collection in foreign countries with even limited logistical support are advised to consult the recommendations of Mori and Holm-Nielsen [17] for a botanical perspective. Additional useful addresses are contained in Davis et al. [18].

3.1 Aspects of Preservation and Molecular Degradation

When obtaining tissue samples, regardless of the study or molecule of interest, the goal should be to acquire the freshest, or best-preserved, samples possible. Denatured proteins and degraded nucleic acids present the single greatest obstacle to a successful molecular research programme. For example, PCR amplification of DNA from old or poorly preserved tissues can be hindered because various forms of damage reduce the average length of intact template molecules for the polymerase enzyme [19, 20]. Damage to the templates may cause the polymerase to stall, thereby

retarding the initial rounds of amplification [21]. The average length of the DNA from ancient soft tissue is less than 200 base-pairs (bp) [22]. This reduction in length is partially attributable to strand breakage caused by autolytic processes (e.g., DNAse activity) that occur rapidly after death [19, 23, 24]. However, equally important sources of damage are subsequent oxidative and hydrolytic effects that either break, or labilise, phosphodiester and carbon-nitrogen (sugar-base) bonds [21, 25, 26]. Although desiccation appears to place a limit on endogenous hydrolytic damage, oxidative attack continues with time.

In an effort to minimise the processes of denaturation and degradation, preservation methods must aim to maintain the tissue samples at low temperature, exclude light and other forms of radiation, remove water and oxygen and sterilise against micro-organisms. These objectives are accomplished by freezing, desiccation or the addition of preservative fluids, and by storing samples in the dark, at constant, low temperature.

3.2 Field Storage of Tissue Samples for Molecular Studies

3.2.1 Live animal specimens

Fresh material consistently provides the highest yield and quality of DNA for amplification, restriction digestion and isozyme analysis. Field trips of short duration are amenable to the collection of live invertebrates and small vertebrates (e.g., herpetofauna), which can usually survive for several days if kept cool, well ventilated and sufficiently humid (dehydration is the primary cause of death in terrestrial taxa). Containers should be kept away from bright sunlight, inside a Styrofoam box and specimens checked twice daily (morning and evening) throughout the duration of the trip. At this time, moisture sources should be replenished, excreta removed and individuals in poor condition preserved immediately in ethanol. Rare specimens should be preserved on collection, rather than kept alive, to safeguard against death going unnoticed during the course of the trip, resulting in inadequate preservation.

3.2.2 Fresh plant samples

Contrary to earlier reports (e.g., [3, 4]), most fresh plant tissue need not be immediately placed on ice or frozen. A diverse array of plant tissues can be kept in a stable condition in Ziploc bags, stored away from light and fluctuating temperatures, inside a Styrofoam box [2]. If collected as whole plants, leaf or shoot cuttings, and placed in bags, leaf tissue is maintained the longest,

provides an immediate voucher when some of the leaf tissue is separated (see below) and allows for subsequent propogation in the greenhouse.

Ziploc bags are ideal for maintaining leaf or shoot tissues in good condition without the addition of moisture (since the tissues will transpire naturally). The addition of a damp paper towel to increase the moisture content of the atmosphere is not recommended, as it can promote waterlogging and rotting of delicate leaves [2].

A small amount of wet ice, placed inside the Styrofoam box containing the bagged samples, is recommended if the trip is of short duration. Once a continuous source of refrigeration is available, the tissue samples should be kept in the refrigerator until processed or placed in long-term ultracold storage (see below). To prevent DNA degradation of fresh tissues, it is important to avoid fluctuating heat exposure or warm up from cold temperatures. The sensitive nature of protein activity in isozyme analysis usually requires that fresh tissues be rapidly exposed to cooler temperatures. However, adequate isozyme activity and high molecular weight DNA have been obtained from samples collected 8 days before being placed in ultracold storage [27]. In some taxa, senescence occurs after collection, during which many proteins disappear from leaves with seasonal aging [28]. It is therefore important to record the age of the leaves at harvest. Seeds, pollen and fern spores should be harvested only when mature. The collector should also be aware that hot and dry weather prior to harvest might cause synthesis of "storage proteins" in seeds and tubers to cease prematurely.

3.2.3 Frozen samples

In comparison with plants, field-collected tissue samples from animals, especially vertebrates, have traditionally been stored frozen, using combinations of wet ice, dry ice and liquid nitrogen. An extensive discussion of methods can be found in Dessauer et al. [3, 4, 28], Simione and Brown [29] and Simione [30]. Although cryopreservation remains important for studies involving proteins and RNA (see below), researchers are increasingly adopting alternative strategies for field-storage of animal tissues.

There are several reasons for this shift in protocol. First, cryopreservation in the field involves considerable planning and logistical support, since sources of dry ice and liquid nitrogen are required. Although dry ice is generally available from airlines, whereas liquid nitrogen can be obtained from medical and veterinary clinics, universities and hospitals, among others (refer to the list provided by Dessauer and Hafner [31]), field trips must be planned to intercept such sources at regular intervals. This may be impractical when trips take place in remote locations or

are prolonged in duration. Second, air transportation of dry ice and liquid nitrogen is strictly regulated (see below). Finally, many investigators have obtained suitable yields of high molecular weight DNA, nearly comparable with those obtained from frozen samples, using less elaborate protocols (e.g., preservation in 95–100% ethanol).

If frozen tissues are to be collected, they should be sampled while the specimen is alive or as soon after death as possible and rapidly placed in the cold and away from light. Tissues should be packed in plastic cryotubes (e.g., Nunc), Ziploc bags, or wrapped tightly in aluminium foil, excluding as much air as possible. Small blood samples can be collected in heparinised hematocrit or microcentrifuge tubes, whereas larger samples are most efficiently collected from heart or caudal vessels using a heparinised syringe [3, 4, 28]. Blood cells should be separated from plasma, prior to freezing, by using a commercial hand centrifuge or a lightweight, plastic centrifuge (e.g., [32]).

As soon as possible after collection and packaging, tissues should be quick (snap) frozen by dropping directly into liquid nitrogen or covering with dry ice. However, since quick freezing often shatters hematocrit and microtubes filled with liquids, such tubes should be frozen slowly in a freezer (if available) before subjection to ultracold temperatures.

For cryopreservation of vegetative plant tissues (mainly leaves), material should be washed in distilled water and quick frozen in liquid nitrogen for subsequent storage at ultracold temperatures. Dessauer et al. [28] provided the following protocol for cryopreservation of seeds: remove fleshy portion of seed; dry seed; place seed in vacuum-sealed container; store in the dark, below 0 °C. Seeds stored in this manner have remained viable for up to 10 years. Pollen and fern spores may be similarly treated (but must first be cleaned of debris and treated with organic solvents to remove lipids) and have yielded stable proteins after 4 years' storage at ≤ –30 °C.

3.2.4 Cell lines

Cryopreservation of living cells requires special collecting, freezing and storage procedures if cells are to survive the freezing and thawing process [33]. Few cells will survive freezing and thawing without a cryoprotectant, e.g., glycerol or dimethylsulphoxide (DMSO). Each species, tissue and freezing system has an optimum cryoprotectant concentration and freezing rate, which must be ascertained to recover the maximum number of viable cells from the sample [34]. Cryoprotectant concentration must be sufficient to protect the cells from freeze damage, yet dilute enough to avoid chemical injury to cells, while the rate of freezing should also be precisely controlled, depending on the species, size of sample, cryoprotectant and freezing system. Rapid

freezing induces death by the formation of crystals within the cells, while slow freezing causes death from the chemical consequences of solute concentration. For further guidelines on the field preservation of cell lines, refer to Hay and Gee [33] and Dessauer et al. [3, 4].

3.2.5 Desiccated samples

Rapid desiccation is one good alternative to cryopreservation for storing tissue samples in the field and may be preferred because it requires neither refrigeration nor flammable substances. Many proteins in carefully dried tissues are stable for short duration at ambient temperature and for longer periods in refrigerators or freezers. For example, acetone powders, solids precipitated from tissues with cold acetone, retain sufficient enzymatic activity for use in endocrinological studies [28]. Desiccated tissues are also suitable for subsequent extraction and PCR amplification of high molecular weight DNA. Specific protocols for the storage of desiccated samples are provided in Protocol 1.

Protocol 1 Methods of desiccation

1. *Insects.* Air-dried, pinned insect samples are convenient, require low maintenance and are lighter than fluid-preserved samples. Insects may also be preserved in ethanol or acetone and later desiccated using a critical point drier [35–37]. Both techniques are suitable for DNA extraction, but marked degradation may occur if tissue dehydration is prolonged (e.g., in humid climates or with large specimens). Placing samples in contact with silica gel for at least 12 hours (determined by the size of the specimen) is recommended in this case [38]. Removing the head, legs, wings, etc. enhances desiccation of large specimens.

Various methods of chemical desiccation, e.g., hexamethyldisilazane (HMDS), amyl acetate, xylene, methyl cellusolve and acetone vapour, provide an alternative to air desiccation, especially in humid climates, yielding high molecular weight DNA amenable to PCR [36]. Specimens can also be dried from ethanol using chemical techniques. Phillips and Simon [39] presented a protocol for the isolation of DNA from pinned insects without destruction of the exoskeleton.

2. *Parasites.* Toe et al. [40] evaluated methods for the field preservation of parasite and vector samples (e.g., *Onchocerca volvulus* and *Simulium damnosum*) for PCR. Preservation of desiccated tissues from parasites and their associated vectors on microscope slides yielded the greatest quantity of high molecular weight DNA.

3. *Vertebrates.* Blood samples may be spotted on Guthrie cards or on Whatman paper (3 mm) and left to dry in bright sunlight for 30 minutes. Makowski et al. [41] recommended usage of guanidium thiocyanate-impregnated filter paper (GT-903), which binds PCR inhibitors and preserves DNA in an aqueous extractable form. If GT-903 is unavailable, consult Ostrander et al. [42] and McCabe et al. [43] on methods for extracting DNA from regular paper. Weisberg et al. [44] found that DNA extracted from lyophilised (freeze-dried) blood was similar in length to that extracted from fresh or frozen blood and suitable for PCR.

Sodium chloride (NaCl) can be used as a desiccant for solid vertebrate tissues, e.g., muscle. Approximately 1 g of tissue should be sliced off and centered in a 15 ml or 45 ml conical test tube containing NaCl. Upon return to the laboratory, the tissues need only be rinsed under distilled water before commencing with DNA extraction. PCR from such samples has resulted in mtDNA fragments up to 300 bp in length [45]. However, the success of this method for isozyme analysis has not been established and is probably unreliable.

4. *Plants.* Plant tissues are often difficult to preserve for molecular studies, due to the presence of phenols, polysaccharides and lipids. Methods of dehydrating plant tissue prior to transport include lyophilisation, dehydration with a food drier, air-drying and herbarium specimen (forced air or heat) drying [46–54]. However, these techniques cannot be universally applied to plants and should be tested before embarking on a field trip [55]. For example, lyophilisation often leads to decreased protein activity, although animal and fungal tissues are usually unaffected [56]. DNA extracted from dried plant tissues is often too degraded for use in restriction site analysis, although successful amplification may be achieved with PCR.

Desiccation of plant tissue in Dierite ($CaSO_4$) or silica gel offers the best alternative to collection of fresh or frozen plant tissue [55, 57, 58], having been successfully tested by extraction and sequencing of DNA, and by digestion with restriction enzymes, over a diversity of angiosperm taxa [2, 53]. It also appears to be less likely to damage proteins than other methods, e.g., lyophilisation [59]. Since the method is simple, the chemicals readily available and easy to transport, it is ideal for obtaining species that exist in remote areas or are to be collected by colleagues in other countries.

Silica gel is a more efficient desiccant than Dierite as it can be obtained in smaller mesh size (28–200, grade 12 from Fisher Scientific, Chicago, IL), allowing for greater surface coverage of leaf tissue. Usually 4–6 g of fresh leaves are placed in small Ziploc bags. Leaf tissue dries faster and thus with less DNA degradation, if first torn into smaller pieces. Subsequently, 50–60 g of

silica gel (minimum 10:1 gram ratio of silica gel to leaf tissue) is added to the bag. Drying should take place within 12 hours, determined if tissue snaps cleanly when bent. Longer exposures to silica gel may be required, e.g., in monocots, but will usually result in DNA degradation. The dried tissue in Ziploc bags should be stored with trace amounts of indicator silica gel (6–16 mesh, grade 42), which changes colour from violet-blue to whitish pink when hydrated, to verify that rehydration has not occurred. The Ziploc bags should, in turn, be kept in tightly sealed plastic boxes. Schierenbeck [60] provided a modified polyethylene-glycol DNA extraction protocol for silica gel-dried plants.

5. *Marine algae.* Holzmann and Pawlowski [61] obtained high molecular weight DNA from air-dried samples of foraminifera stored at ambient temperature for up to 11 weeks. Positive results were also obtained with foraminifera stored for 2–3 years, although amplification products were < 500 bp long.

6. *Marine invertebrates.* Desiccation is generally not viable for marine invertebrates (e.g., Scyphozoa, Polychaeta) because their high water content is incompatible with the requirement that samples be dried within 12 hours to prevent DNA degradation [55].

3.2.6 Fluid-preserved samples

Tissue samples from invertebrates, intended for molecular studies, are routinely stored in 95–100% ethanol at ambient temperature (e.g., [62]). Preservation in methanol and propanol is contraindicated [38], although tissue samples preserved in 100% methanol will yield higher molecular weight DNA than samples preserved in formalin [63].

Greer et al. [64] demonstrated that only storage in 95–100% ethanol (v/v) results in PCR products of 1–2 kb after 30 days. Adequate preservation in 70–80% ethanol may occur if specimens or tissue samples are small and, in the case of arthropods, weakly sclerotised, but DNA isolated from larger specimens preserved in 70% ethanol is usually highly degraded. For large or heavily sclerotised samples, even 95–100% ethanol is no guarantee against degradation, because saturation of the tissues may be delayed by the size or impermeable nature of the tissues [65]. Such samples should be injected with ethanol, dissected into smaller pieces or, in the case of arthropods, cut in several places along the exoskeleton, to allow the ethanol to diffuse directly into the internal tissues. Excess ethanol (i.e., a high ratio of ethanol to tissue sample volume) should always be used when preserving samples for molecular studies, to minimise dilution that

occurs with addition of the sample [66]. Ethanol should be replaced after the initial fixation and periodically thereafter. Initial fixation of fresh or frozen tissues at –20 °C has been found to contribute significantly to the quality of tissue preservation in arthropod samples [67], but refrigeration is seldom available in the field.

The method of euthanasia may also affect the preservation of tissues, especially if whole organisms are placed directly in ethanol. Animals as diverse as nematodes and scorpions are prone to close their oral, anal and respiratory openings on placement in ethanol, thus further hindering ethanol diffusion into the internal tissues after death. Such animals should be frozen alive, placed in warm ethanol (unless being used for isozyme analysis), very dilute (e.g., 10%) ethanol, or dissected/cut (after euthanasia with ethyl acetate, chloroform or cyanide), prior to placement in 95–100% ethanol. Alternatively, lysis buffers may be used for their preservation (see below).

Ethanol is suited to the storage of vertebrate tissues and has been used successfully in DNA hybridisation and sequencing. Solid tissues, e.g., muscle, should be cut into pieces approximately 10 mm in diameter to allow rapid penetration of ethanol. After saturation in ethanol for at least two days, moist tissues may be transferred to plastic bags for storage or shipment [28].

Fungi and marine algae may also be stored in ethanol. Ethanol-preservation was widely considered ineffective for maintaining adequate yields of high molecular weight DNA from land plants [51] until Flournoy et al. [68] obtained excellent yields from samples stored in 95–100% ethanol or 100% methanol by addition of proteinase (Pronase E). Vacuum infiltration of ethanol resulted in better DNA preservation than passive infiltration [68].

In the absence of ethanol, most samples may be stored in saturated salt or buffer solutions until transported to laboratories equipped with appropriate resources. Even laundry detergent has proven to be a rapid and uncomplicated temporary storage solution for recovering high yields of DNA [69, 70].

Many of these buffer solutions are also used for DNA isolation and may be advantageous for the preservation of highly sclerotised organisms. For example, Sansinforiano et al. [71] used lysis buffer with a high urea concentration for preserving the impermeable mucopolysaccharide capsules of *Cryptococcus neoformans* and other pathogenic yeasts, and acquired high molecular weight DNA after storage at ambient temperature for up to 6 months. Details of these methods are listed in Protocol 2.

Protocol 2 Preservation in buffer solutions

1. *Blood.* Preservation of vertebrate blood samples in the field has traditionally employed buffer solutions, e.g., 2% 2-phenoxyethanol with glycerol or DMSO, 2-propanol with ethylene-diamine-tetraacetate (EDTA) or sodium dodecyl sulphate (SDS). Such buffers can preserve blood proteins (e.g., plasma albumin) and mRNA for up to three weeks without refrigeration [72, 73], but must usually be frozen within 24 hours of collection [28].

Quinn and White [74] recommended that blood samples from birds, injected into 5 ml vacutainer/EDTA tubes, should be frozen within 10 hours of collection to prevent significant DNA degradation. However, Cann et al. [45] obtained high molecular weight DNA from unrefrigerated blood samples of birds, bats and wallabies, using a scaled-down version of Quinn and White's protocol.

Cann et al. [45] reported that microhematocrit tubes of blood blown into 500 µl of TNE_2 (10 mM tris-hydroxymethyl amino methane, 10 mM NaCl and 2 mM EDTA, pH 8.0) are stable for months if refrigeration is impossible and still provide high molecular weight DNA. The use of mannitol-sucrose buffer in mtDNA studies is also reported to be adequate for storing samples up to 2 weeks without refrigeration [75]. Gelhaus et al. [76] provided protocols for the isolation of DNA from urea-preserved blood. For practical guidelines on the extraction of blood samples from vertebrates, refer to Dessauer et al. [3, 4].

2. *Vertebrates.* The lenses of vertebrate eyes, collected for sequence studies of alphacrystallin, have been preserved in saturated guanidine hydrochloride [28] and this solution is also purported to be effective for the preservation of proteins in other vertebrate tissues [45].

High molecular weight DNA has been extracted from vertebrate solid tissue samples, stored at ambient temperature in 4 M guanidium isothiocyanate (GITC) from 41 days [77] to 3 months [78]. Vertebrate tissue samples may be kept up to three years without refrigeration in TNES-urea (6 or 8 M urea; 10 mM Tris-HCl, pH 7.5; 125 mM NaCl; 10 mM EDTA; 1% SDS) and still yield high molecular weight DNA [79].

3. *Invertebrates.* Sperm has been stored in 0.01–0.02% sodium azide solution [14]. Laulier et al. [77] extracted high molecular weight DNA from field collected samples of viruses, bacteria, yeasts and invertebrates stored up to 41 days at ambient temperature, in 4 M GITC.

Dawson et al. [80] assessed the effects of five buffer solutions (70% ethanol, Queen's lysis buffer [0.01 M Tris, 0.01 M NaCl, 0.01 M disodium-EDTA and 1.0% n-lauroylsarcosine, pH 8.0], DMSO-NaCl solution, CTAB-NaCI solution, and a urea extraction buffer) on the preservation of marine invertebrate samples for DNA isolation. In accord with Seutin et al. [81],

these authors concluded that dimethylsulphoxide and sodium chloride (DMSO-NaCl) was the best solution in which to store marine tissue samples. Reiss et al. [65] recommended that insect samples be thoroughly homogenised in order to achieve adequate DNA preservation in buffer solutions.

4. *Plants*. Samples of plant tissue preserved using cetyltrimethylammonium bromide (CTAB) yield high molecular weight DNA, thereby providing an alternative to standard desiccation methods [82].

3.2.7 Embryonic tissues

Developmental data are becoming increasingly important in modern evolutionary and systematics studies [83, 84]. Developmental studies utilise embryonic tissues that hold expression data (transcribed mRNA for *in situ* studies and translated proteins for antibody staining approaches), the preservation of which is essential. Accordingly, embryonic expression work is best conducted with live material. This can be obtained from embryos collected in the field or from sources of live organisms listed in the next section.

Knowledge of embryogenesis in the organisms of interest can aid in the collection and treatment of embryos in the field. For instance, if an organism displays brooding behaviour, large numbers of embryos can be obtained by locating reproducing individuals [85, 86]. Such individuals can be brought alive to the lab and manipulated for fixation.

If live material cannot be transported to the laboratory, embryos may be preserved in the field, provided that the preservation of mRNA and proteins can be assured. Although embryos can be frozen at –80 °C with liquid nitrogen, fixation methods are easier and more efficient for their preservation in the field. However, knowledge of the embryology of organisms is required, because fixation of embryos without certain kinds of pretreatment may render them unusable. For example, *Drosophila* embryos require dechorionation prior to fixation. Such pre-fixation treatments may be simple to accomplish in the field. Alternatively, if embryonic tissues like imaginal discs in insects are the targets of molecular developmental research, whole larvae can be preserved in fixatives to allow later dissection of tissues for whole mount or sections. Specific protocols for preservation of embryos in the field are provided in Protocol 3.

Protocol 3 Storage and fixation of embryos

1. *Dechorionation.* This procedure is accomplished by treating embryos in sodium hypochlorite followed by thorough washing in water or 0.7% saline. This step can be accomplished in the field with test tubes and a small supply of Pasteur pipettes. Other pre-fixation treatments may be necessary to prepare the embryos or developing organisms for fixation or the researcher may determine that pre-fixation treatments are unnecessary and proceed directly to fixation of materials.

2. *Fixation.* Dechorionated embryos or untreated embryos can be fixed by several methods depending on whether antibody staining or mRNA *in situ* detection is the goal. Antibody staining requires that larvae be fixed in a solution of 4% formaldehyde in 50 mM cacodylic acid (pH 7.4) for 1 hour at 20 °C, dehydrated in an ethanol series and stored in methanol at –20 °C. *In situ* hybridization requires that larvae be fixed in a solution of 4% formaldehyde in PBS, dehydrated in an ethanol series and stored in methanol at –20 °C.

3. *Storage.* Fixed embryos should be transferred as quickly as possible to a –20 °C freezer, where they will remain for long-term storage.

3.2.8 Fecal samples

PCR amplification of DNA from fecal samples is dependent on preservation method, PCR-product length and whether nuclear or mtDNA is assayed. Storage in DMSO/EDTA/Tris/salt solution (DETs) is most effective for preserving nuclear DNA, but storage in 70% ethanol, freezing at –20 °C, and desiccation using silica beads perform equally well for mtDNA and short (< 200 bp) nuclear DNA fragments [87, 88]. Protocols for the isolation of DNA from fecal samples stored by these methods are provided by Wasser et al. [87], Frantzen et al. [88], Shankaranarayanan and Singh [89] and Launhardt et al. [90]. Dowd et al. [91] described protocols for the extraction of DNA from formalin-fixed fecal samples.

3.3 Practical Considerations

3.3.1 Contamination

Throughout the duration of fieldwork, collectors should be aware of the importance of keeping their instruments, containers and reagents clean in order to prevent cross-contamination.

Individual tissue samples should be stored in separate containers, if possible, even when the level of analysis to be examined does not require it (e.g., DNA restriction site analysis among populations). Future studies involving single-copy genes, introgression, hybridisation or recombination require knowledge of specific sources of the molecules. If storage of samples in the same container is unavoidable, polyethylene sacks may be used to separate individual samples [92].

An attempt should be made at the outset to remove all dirt and contaminating organisms (e.g., epiphytes, fungi, ectoparasites) from the specimen or tissue sample. It may not be possible to remove all such organisms (e.g., endoparasites and gut flora), but this problem can be circumvented by judicious tissue choice (see above) and the use of taxon-specific primers in the PCR. Mites are a particularly problematic contaminant of field-collected fungal and algal samples. Not only can these arthropods destroy specimens, they can also cross-contaminate a culture collection as they move about [93]. Additionally, the removal of contaminating organisms is important for preventing the introduction of foreign organisms across international borders (see below).

3.3.2 Labelling and documentation

Investigators should meticulously label all materials they collect. Labels should include species (if identified), collection locality, brief habitat description (including reference to collection method used), date of collection, collector, voucher number, etc. [94]. When working in a team, the principle investigator should allocate documentation duties at the outset and assign a backup. All team members should be conversant with the system used to identify and inventory samples that have been collected, including unambiguous abbreviations, the order in which information should appear on the labels and other critical information about the provenance of the sample, which may disappear when field notes are transcribed.

Permanent ink markers should be used for labelling, but must be tested beforehand in water, ethanol and extremes of temperature [95, 96]. Labels should be written directly on the sample bag or container or, if affixed, tied as well as taped, since tape is liable to come loose during handling, freezing and thawing. Alternatively, labels can be etched into glass or plastic tubes with a diamond-tipped pen [97]. Permanent ink marker or heavy lead-pencil labels, written on roughened paper or card [98, 99] and tied to the specimen itself or placed inside the container, are the safest guarantee that collection and provenance data will remain with appropriate samples.

The use of synthetic, polypropylene paper (e.g., Kimdura or Tyvek, available from Kimberly-Clark, Dallas, TX) prevents tearing of labels [92, 100, 101].

It is prudent to test all vials, tubes or other containers of unknown composition before departure, to ascertain if they are unbreakable, solvent-resistant and leakproof (for fluid-preserved samples) or can withstand ultracold conditions (for frozen samples). Fragile glass tubes (e.g., hematocrit tubes) can be inserted into the slots of corrugated cardboard for protection during storage [45].

Water-resistant pocket notebooks and pens with waterproof (and solvent-resistant) ink are as essential to successful collecting as sterile vials and bags [97]. The use of traditional collector's catalogues (e.g., [102, 103]) organised such that each specimen receives a unique number preceded by the collector's initials, is recommended [3, 4]. Catalogue entries should record the type(s) of tissues to be sampled and catalogue numbers should be recorded on the vials containing tissues.

Modern biorepositories often use standardised specimen vials with bar-coded labels printed on thermal- and solvent-resistant material suitable for long-term storage. Advance planning with regard to tissue voucher disposition may allow a researcher to obtain a series of numbered sample vials from the repository prior to the outset of fieldwork. The researcher may then refer to the vial number in the field notebook with regard to collection data, thereby circumventing the need to label vials in the field and facilitating the access to specimens in the repository and its associated database on completion of the study. Advance knowledge of the repository accession numbers associated with specimens also facilitates the preparation of manuscripts.

Primary documentation in the field will always be done by hand (if for no reason besides the fact that the backup systems for field computers are subject to failure). Nevertheless, notebook computers have provided an additional innovation to the documentation of field data. Battery operation for more than three hours, with one-hour recharge times from 110 V outlets or car batteries, together with the ability to link via internal or facsimile modems to remote laboratory computer facilities, has allowed the maintenance of up-to-date database files [2]. For further suggestions on methods of cataloguing in the field, refer to Baker and Hafner [97] and Dessauer et al. [3, 4].

3.3.3 Hazardous organisms

Preventative measures may be needed to protect the researcher working with certain organisms. Investigators should not attempt to work with these taxa without proper equipment and a

thorough appreciation of the risks involved. For example, investigators working with venomous animals should wear protective clothing and carry antivenom or venom aspirators, in the event of accidental envenomation. These precautions are especially obvious for those involved in the "milking" of venom for their investigations [3, 4].

Investigators working with some mammals and their parasites should be vaccinated against rabies or tuberculosis. Animal necropsy should also include protective clothing and the containment of possible biohazards, in order to avoid contracting diseases such as psittacosis, erysipelas, rickettsial infections and brucellosis [104]. The National Wildlife Health Center (Madison, WI) has a Resource Health Team available to examine animal specimens for possible diseases (Appendix 2).

Some fungi or their secondary products can also cause virulent or chronic disease in animals and may require special procedures [105]. Culture collection staff (see below) may be able to advise on the necessary procedures for pathogenic fungi.

4 Other Sources of Specimens and Tissue Samples

There are many ways in which tissues can be obtained without even going into the field. Stock centers and culture collections, commercial supply companies, cell line centers, seed/spore banks, botanical gardens and zoological parks are the most frequently used sources for fresh tissues. The primary consideration in obtaining tissues from any of these sources is the reliability of the identification of the organisms that are used for the tissues. If the species designation is suspect, the specimen may not be worth the trouble. In addition, the exact origin of organisms from these collections is often unknown or ambiguous, such that caution should be exercised with specimens obtained from these sources if precise location data are needed for a particular study.

In addition to these sources of fresh samples, vast numbers of frozen, desiccated and fluid-preserved samples exist in natural history museums, herbaria and frozen tissue collections. Such samples are generally accurately identified, documented and, if collected in series, vouchered. However, some (e.g., desiccated and fluid-preserved samples) may provide considerable challenges to protein or nucleotide extraction, depending on the method and extent of preservation.

4.1 Sources of Fresh Specimens and Tissue Samples

4.1.1 Stock centers and culture collections

Stock centers exist for several animal taxa (e.g., *Caenorhabditis*, *Drosophila*, *Peromyscus* and *Tribolium*), from which particular strains or species may be ordered at cost (Appendix 3). The services of such centers, including preparation, testing, preservation, maintenance and shipping of the cultures, are expensive, hence user fees must defray costs [106].

Animal stock centers are often limited in the diversity of taxa available, although some hold a large variety. For example, the Jackson Laboratory (Bay Harbor, ME) maintains more than 2500 strains of genetically defined mice for biomedical research, while the *Drosophila* Species Stock Center (Tucson, AZ) can supply approximately 300 species of *Drosophila*. Samples of bacteria, fungi, algae and other unicellular protists may similarly be obtained at cost from culture collections, where they are maintained in pure culture. Lyophilised cultures of fungi may be shipped immediately on order, but algae must usually be grown out on agar or in liquid medium so that shipping may be delayed several weeks.

Detailed information on worldwide culture collections is available from the World Federation for Culture Collections (WFCC) which maintains the World Data Center on Microorganisms (WDCM) and is a component of the UNESCO Microbial Resources Centers (MIRCEN) network. As part of their mission to disseminate information on culture collections, the WDCM has produced two useful publications: the *World Directory of Collections of Cultures of Microorganisms* [107], which includes 345 fungal collections, and the *World Catalogue of Algae* [108]. In addition, users may search the listings of all the collections for individual species on the WDCM electronic database (Appendix 4). According to this database, 472 culture collections in 62 countries are currently registered in WDCM. A guide to the database (*Guide to World Data Center on Microorganisms – A List of Culture Collections of the World*) is also available. The websites of other culture collections and germplasm repositories are listed in Appendices 3 and 4.

In addition to the major collections with broad general holdings, many smaller collections occur throughout the world, which might be of interest for providing samples for particular studies or geographic regions. Medical research institutes, which include departments specialising in parasitology, toxicology and medical entomology (e.g., Instituto Butantan, São Paolo, Brazil; South African Institute for Medical Research, Johannesburg, South Africa), often stock permanent cultures of common medically important local taxa (e.g., mosquitoes, spiders, scorpions, snakes and unicellular protists), from which samples may be supplied for molecular studies, on request. Insect fungal pathogens can be obtained from the specialist collection of entomopathogenic fungi maintained by the U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) at the U.S. Plant, Soil and Nutrition Laboratory (Ithaca, NY). Cultures of

many isolates of wood-rotting basidiomycetes are available from the USDA Forest Service at the Forest Products Laboratory and Center for Forest Mycology (Madison, WI), which maintains voucher specimens for every culture. The *Chlamydomonas* Genetics Center at Duke University (Durham, NC) houses a large collection of *Chlamydomonas reinhardtii* mutants and numerous strains of other *Chlamydomonas* species and provides a printout of file information on each strain. The Soil Microbiology Division of the International Rice Research Institute (Manila, Philippines) maintains a collection of prokaryotic nitrogen-fixing blue-green algae, and a collection of algae with high potential for use in biomass energy production is available at the National Renewable Energy Laboratory (Golden, CO).

Most curators of culture collections ensure that cultures are correctly identified, even returning transferred cultures to the depositing scientist for authentification before cataloguing [106]. Accordingly, the majority of samples obtained from such cultures will be correctly identified (although collection data may be unavailable). Where multiple strains of species are offered by culture collections, informed choices should be made, depending on the research question. Strains may differ in metabolism and other genetic characters, which may be important in selecting a culture. When strains are intended to be representative of the species, so-called type cultures (i.e., those isolated from the type collection of a species at the time of its description) should be ordered preferentially. Two useful publications of the American Type Culture Collection (ATCC) list processes and products associated with many isolates in their collection [109, 110] and additional information may be found on their website (Appendix 4). Similarly, some culture collection catalogues contain information, including literature references to strains [111, 112].

Ploidy number should be a further consideration when selecting fungal cultures. Highly variable DNA regions in single-copy or repetitive genes (e.g., ribosomal RNA genes) may vary at single base positions in the homologous chromosomes of diploid or dikaryotic isolates [113]. Haploid cultures from many fungi with gametic meiosis can be readily acquired from single spores, whereas isolates from basidiomata, ascomata or mass spore cultures of heterothallic basidiomycetes and ascomycetes provide strains with nuclear variation [93]. Diploid material is difficult to avoid in some organisms (e.g., oomycetes), but it is important to recognise the possibility of variation. DNA extraction from single spores [114] may eliminate some ploidy problems and provide a means for a variety of intraspecific studies. The ploidy level of most algae in culture collections is known or can be inferred from the life cycle (information available in general texts such as Bold and Wynne [115]).

4.1.2 Cell line centers

Cell lines offer an alternative source of fresh tissues for comparative research. A large selection of cell lines are held in storage by a variety of different centers (Appendix 4), e.g., viral infected cell lines, cell lines for large mammals and cell lines for human diseases [106, 116, 117]. Further examples of the breadth of taxonomic representation are provided on the websites of the ATCC (American Type Culture Collection) and the ECACC (European Collection of Cell Cultures). The CRES (Center for Reproduction of Endangered Species, Zoological Society of San Diego, CA) supplies a variety of endangered vertebrate cell lines and tissues (Appendix 5).

Many centers store multiple different lines derived from different tissues for a variety of taxa, thus allowing specific tissues to be selected for studies in which tissue specificity is a concern [106]. The major cell line centers such as the ATCC and ECACC are easily accessible via email or their websites, which provide online order forms to facilitate the rapid purchase of cell lines. These centers also offer advice on the materials and methods of cell culturing, once the cell lines have been shipped to researchers. Each cell line center has specific terms of usage that the researcher should consult before placing an order (see below).

4.1.3 Commercial Sources

Live representatives of most animal phyla and pure cultures of some protists can be obtained relatively cheaply from biological supply companies (Appendix 6). Ordering specimens is convenient insofar as they are shipped direct to the laboratory, thereby saving considerable time and effort. However organisms should be ordered as far in advance as possible, since their appearance in the field may be seasonal or unpredictable. Identifications are usually correct (common, well-known taxa are usually stocked), but voucher specimens should still be preserved (see below).

The commercial pet trade provides yet another convenient source of specimens or tissue samples for some animal taxa, notably birds, fish, herpetofauna and selected terrestrial and marine invertebrates. Prices of commonly available species acquired from these sources are usually reasonable, but less commonly available taxa may be expensive. Moreover, although certain common or captive-reared species are available year-round, the availability of most field-collected species is highly unpredictable and requires constant vigilance on the part of the investigator. Apart from the commonly available species, identifications of most specimens acquired through the pet trade should be viewed with suspicion, especially where reptiles, amphibians and invertebrates are concerned. Besides general identification errors made by

untrained individuals (especially common with sexually dimorphic taxa), deliberate misidentification is used by certain dealers to enhance the apparent "diversity" of taxa on offer and hybrids are often sold as "new species." Collection data, if available (seldom more than country of origin, which is acquired third-hand by the dealer from the importer, who received it from the collector!), should be mistrusted for the same reasons. Finally and most importantly, it behooves the investigator intending to use the pet trade as a source of specimens to ensure that the specimens to be purchased were acquired legally by the dealer (addressed further below).

4.1.4 Botanical and zoological gardens

Botanical gardens (including arboreta and university greenhouses) are an established source of land plant tissue, whereas zoological gardens (including aquaria, aviaries, butterfly gardens and snake parks) are just emerging as a source of tissue from vertebrates (and certain invertebrates). Currently, CRES is the only zoological institution to make their resources formally available. The large diversity of plant and animal families and genera maintained by botanical and zoological gardens is ideal for higher level molecular studies using exemplar taxa. More specialised collections (e.g., certain arboreta, butterfly gardens and snake parks) may also be useful for molecular studies at the genus or species level. Most directors of botanical or zoological gardens are very willing to provide access to their collections for molecular study as it enhances the role, and thus continued support, of the garden. However, since animal tissues may have to be sampled when animals are sedated for medical examination, investigators should request samples well in advance.

Despite the obvious benefits of obtaining tissue samples from botanical or zoological gardens (large diversity, ease of collection and transport, saving in cost and time), potential problems remain. Foremost is the lack of complete voucher information (collector, date and exact locality), attached directly or indirectly in the records, to the specimen. A more serious problem is the possibility of misidentifications and label switches, although these can be obviated by making vouchers for subsequent identification by specialists in each group. A further problem concerns collection and transport of the material. Many gardens have neither time nor personnel to oversee the multitude of requests for shipment of samples and should not be expected to bear the associated expenses. Personal contact with scientists and managers at the gardens is recommended.

Henderson and Prentice [118] provide a worldwide listing of botanical gardens. Various other sources, e.g., the teaching guide to the text *Biology* [119], also contain extensive lists of zoos and botanical gardens. Recent listings can be found at the websites provided in Appendix 7.

4.1.5 Seed and spore banks

Seed banks are becoming an increasingly important source of plant tissue in molecular studies, especially where agronomically important groups, including cultivated species and their wild relatives (e.g., *Brassica*, *Solanum*, *Triticum*, *Zea*, *Glycine*), are involved (Appendix 3). The U.S. National Plant Germplasm System (NPGS), coordinated by the National Germplasm Resources Laboratory (Beltsville, MD), comprises 22 repositories that are annually updated and planted out to maintain their collections. One of these, the U.S. Potato Gene Bank (Sturgeon Bay, WI), was essential for a large molecular research program on the cultivated potato and its wild relatives [2]. Besides maintaining an extensive collection of seeds, the National Seed Storage Laboratory (Fort Collins, CO), another repository of the NPGS, is investigating pollen storage as a means of storing germplasm of clonally held species [120].

In addition to such formal repositories, many botanical gardens maintain seed exchange lists (e.g., *Index Seminum* of the Modena Botanical Garden, Italy). The Millenium Seed Bank Project of the Royal Botanical Gardens, Kew has the most extensive listing of fully documented plant species, including many families of noncultivated plants.

Although fronds and rhizomes are most often used as tissue sources from ferns or fern allies, spores and resultant gametophytes are equally suitable. The American Fern Society maintains a spore exchange program and information can be obtained from the *Bulletin of the American Fern Society* or their website (Appendix 3).

Potential problems with seed or spore sources, whether from seed banks or university greenhouse collections, include contaminated seed, errors in handling and labelling, and misidentification. Such problems can be avoided by vouchering all plant tissue grown from seed.

4.1.6 Gifts or exchanges with scientific colleagues

A commonly used alternative source of field-collected material is through gifts or exchanges with colleagues in foreign countries, who are either specialists in the group under study or knowledgeable about specific collection sites. Although the use of local scientists can add greatly to the breadth of the study, several practical issues remain, including the provision of detailed

instructions concerning shipment of material and the necessity for providing permits if crossing international borders (see below). A further issue to consider is whether the support in obtaining samples from colleagues has been so extensive and critical to the success of the project that nothing short of co-authorship would be adequate compensation.

4.2 Frozen Tissue Collections

Many natural history museums have created, and continue to support, frozen tissue collections or collections that harbor tissues in ethanol or lysis buffers [121, 122]. Two directories of frozen tissue collections ([4, 31], reproduced and updated in Appendix 5) list public and private institutions in the U.S.A. and abroad that have holdings of frozen tissues. Several frozen tissue collections are now online while others are in the process of producing websites for easy electronic access via the internet.

Some requests are easily fulfilled by these facilities, as with commonly available tissues of which large quantities exist in storage. Other requests are more difficult to fulfill, as with tissues of endangered species of which precious little remains. Consequently many frozen tissue facilities require a formal application from the researcher wishing to obtain loans, especially for the more difficult requests (for examples of frozen tissue collection policy, refer to the websites in Appendix 2). First, the loan request must be accompanied by some assurance that the applicant is proficient in isolating the molecules of interest from frozen specimens [97, 123]. Such assurance is usually demonstrated in a short proposal that the applicant submits to the repository for curatorial approval. Second, many frozen tissue facilities request that a proportion of the DNA or proteins isolated from the frozen tissue be deposited back in the frozen tissue facility in return for the loan.

Shipments of frozen tissues are costly to package and transport hence payment of tissue shipment costs is normally requested of the recipient [97]. Investigators planning to obtain samples from frozen tissue collections should provide for these costs in their grant budgets.

4.3 Sources of Desiccated or Fluid-preserved Specimens and Tissue Samples

4.3.1 Natural history museums

Museum collections represent a tremendous resource for molecular studies. The world's research collections contain vast amounts of material amenable to molecular analysis that may be

otherwise unavailable due to extinction or collection difficulties. This can be especially acute in higher level studies of geographically diverse taxa. Such collections can make possible, in time and resources, studies for which it would take years to gather material.

Zoological collections are composed predominantly of skeletal remains, dried skins, dried insects and fluid-preserved specimens. Skeletal material, which makes up a large proportion of vertebrate collections, is usually devoid of amplifiable DNA [19, 21, 22, 124]. Nevertheless, Hagelberg et al. [125] amplified DNA from human bone samples 300–5500 years old. More recently, methods developed for protein extraction from ancient bone were found to yield large amounts of DNA with molecular weights much higher than those seen from ancient soft tissue [126–128]. Dried, untanned skins routinely yield DNA sequences, as do dried, pinned insects, although the DNA may be extremely degraded [38, 64, 124].

The vast majority of helminth and arthropod specimens are preserved in ethanol, in which DNA preserves fairly well (depending on collection practices), although it is usually degraded to < 2 kb, especially if 70% ethanol was used, as is prevalent in older collections [65]. However, other fixatives, e.g., glycerin and 2-propanol (in arachnid collections), may have been added to the ethanol in an effort to harden, soften or maintain the colour of the specimens. For example, terrestrial planarians, stored long-term in 80% ethanol, must first be fixed in a formaldehyde calcium cobalt solution [62].

Fluid-stored insect collections may contain similar fixatives, including formamide, picric acid, formalin and glycerin. Any of these fixatives may likewise affect DNA, hence it is crucial to know how samples were collected and maintained. For example, DNA isolated from insects preserved in acetone, ethyl acetate, formal saline, Carnoy's solution (ethanol:acetic acid, 3:1), methanol, or propanol was highly degraded, compared with that extracted from insects preserved in liquid nitrogen, preserved in ethanol (stored at 4 °C or at ambient temperature), sun-dried, or dried over silica gel [15, 38, 65].

Vertebrates and marine invertebrates (e.g., cnidarians, ctenophores and ctenostome bryozoans) have traditionally been preserved in solutions of 10% buffered-neutral formalin (BNF) (v/v) with implications for DNA isolation from such samples. PCR is greatly affected by the amount of time spent in formalin, the temperature at fixation and the method of buffering. For example, Greer et al. [64] found that fragments of DNA, approximately 1 kb in length, were impossible to amplify from formalin-fixed tissues after only 24 hours at ambient temperature. However, high molecular weight DNA was obtained from tissues fixed at 4 °C in formalin buffered with 4 M urea [23, 129]. Similarly, Noguchi et al. [63] established that fixation in formalin at 4 °C and in formalin containing 5 mmol/L EDTA at ambient temperature preserved significantly more high molecular

weight DNA than fixation in formalin at ambient temperature. Savioz et al. [130] recently provided protocols for the isolation of high molecular weight DNA from tissues stored in formalin for nearly 50 years. A simple test for the identification of formalin *versus* ethanol-preserved specimens, based on a colour change in the acid-base indicators sodium sulphite and sodium metabisulphite, was reported by Waller and McAllister [131]. Tests for other fixatives were outlined by Moore [132].

Greer et al. [64] and Criscuolo [133, 134] evaluated the ability of various other fixatives, traditionally used in vertebrate collections, to yield DNA fragments of moderate size on PCR amplification. Most fixatives gave poor results, e.g., acetone, Zamboni's solution, Clarke's solution, methylated spirits, phenol, chloroform, glutaraldehyde, paraformaldehyde, formalin-ethanol-acetic acid (FAA) and metacarn. The worst results were obtained with highly acidic fixatives such as Carnoy's solution, Zenker's solution and Bouin's solution. Zenker's solution is a common tissue fixative (5 parts glacial acetic acid mixed with 95 parts saturated mercuric chloride and 5% potassium dichromate) which affects the stability of DNA in tissues preserved in it, owing to high heavy metal concentrations which promote phosphodiester breakage. Bouin's solution was formerly the fixative of choice for many studies of infectious or parasitic diseases in animals, where a large piece of tissue was preserved in excess solution. However, parasitic samples preserved even under these conditions may be useful if investigators design PCR primers to yield small (< 200 bp) fragments.

Overall, most of the wet and all of the dry samples in museum collections are potentially useful to molecular studies. Given the resources of some of the large institutional collections, some intractable problems may be resolved and some groups whose molecular biology is poorly known may be sampled more completely (e.g., [135]). Readers should consult the *Ancient DNA Newsletter* for updates on protocols suitable for the successful isolation and amplification of DNA from museum specimens.

4.3.2 Herbaria

As with museum collections, there is increasing interest in herbaria because they can potentially provide DNA from identified, fully vouchered species, including types and species that are rare or nonculturable [136–138]. Like museums, the role of herbaria will undoubtedly become more important as PCR and sequencing technology allows more rapid and extensive surveys at all taxonomic levels.

Herbarium specimens of fungi as old as 50 years [139] and nearly 100 years [140] have provided adequate templates for PCR of ribosomal genes. Several studies have also successfully extracted DNA from plant [48, 50, 51] and algal [141] specimens, which similarly proved to be good templates for PCR.

Douglas and Rogers [142] assessed the effects of seven cytological fixatives (3.7% formaldehyde at pH 3.0 and 7.0, FAA at pH 3.0 and 7.0, 1% glutaraldehyde at pH 3.0 and 7.0, and Lavdowsky's fluid, containing mercuric chloride, at pH 3.0) and one storage buffer (SED = NaCl-EDTA-DMSO, pH 7.0) on the DNA of nine plant and fungal species. DNA from untreated tissue and SED-treated tissue was of high molecular weight (> 50 kb). DNA from glutaraldehyde-treated tissues averaged 20 kb in length, while DNA from all other treatments averaged < 8 kb in length. Nearly all attempts to amplify from specimens treated with 3.7% formaldehyde (at pH 3.0 and 7.0) failed.

Although DNA from desiccated herbarium specimens may be partially degraded, primer-directed amplification, especially of multiple copy genes, can overcome the problem. However, the relatively poor quality precludes efficient restriction site analysis [55]. Generally, specimens fast-dried at moderate temperatures, or the edges of large specimens, provide the least-degraded DNA [93]. It is unlikely that isozymes will ever be routinely extracted in an active state from herbarium specimens, although certain desiccated tissues can provide good activity for some systems [58], discussed above. In addition to providing DNA from tissue, herbarium specimens of fungi and algae may contain viable propagules for establishing cultures [143] or extracting DNA [141]. Selected websites, containing recent listings of natural history museums and herbaria, are provided in Appendix 8.

4.3.3 Medical facilities

Archival collections of paraffin-embedded tissues prepared for histology [144–146] and collections of dried blood spots on Guthrie cards stored by state neonatal-screening laboratories [41, 147, 148] represent valuable resources for retrospective studies in clinical pathology and molecular epidemiology (Appendix 4).

Protocols for DNA extraction from paraffin-embedded tissues up to 40 years' old were provided by Frank et al. [149], Morgan et al. [150] and Pavelic et al. [151]. Pavelic et al. [151] assessed the effects of different fixatives on paraffin-embedded tissues, noting that 10% formalin caused irreversible DNA damage that was greater with prolonged fixation time, while tissues stained with Carnoy's, AmeX and Papanicolaou fixatives resulted in consistent yields of high

molecular weight DNA. Makowski et al. [41], Ostrander et al. [42] and McCabe et al. [43] provided methods for extracting DNA from blood spots on paper.

5 Transportation, Long-term Storage and Archiving of Specimens and Tissue Samples

Whether specimens and tissue samples are collected in the field or acquired from other sources, they will have to be transported back to the laboratory for processing. Transportation methods must aim to retain the samples as intact as possible during transit, which may require rapid delivery and be subject to a variety of regulations, depending on the nature of the samples (e.g., live *versus* preserved) and the mode of shipping (e.g., airline, courier, mail).

On arrival in the laboratory, samples must be processed (including identification, if this has not already been conducted), excess tissues stored for future use, voucher specimens retained (or deposited in appropriate institutions) and collection data archived in a computerized database. A variety of approaches to long-term storage and archiving are available to the investigator, the choice among which depends both on the taxa and molecules of interest.

5.1 Transportation

Protocol 4 lists considerations relevant to the transportation of a variety of preserved specimens and tissues. Specific recommendations for packing and labelling are provided in Protocol 5.

Protocol 4 Common transportation methods

1. *Live specimens and cultures.* Invertebrate material is best shipped live from supply houses. However, transit times and export/import regulations may prevent this option when international shipping is involved. Small insects such as *Drosophila* or *Tenebrio* can be sent in vials containing limited amounts of food. Larger arthropods (e.g., scorpions) and many reptiles will tolerate several days without food or water. Most freshwater and marine organisms (adults or larvae) can be shipped live in sealed plastic bags.

Packages containing live specimens should be well padded, slightly humid and insulated with Styrofoam against external temperature fluctuations. Live field-collected invertebrates may also

be transported as airline hold or carry-on baggage, again subject to the necessary export and import permits for live animals and the airline regulations.

Algal (and occasionally fungal) samples are usually provided from culture collections as liquid- or agar-grown cultures and may require specific light or temperature requirements during shipping. Although most phototrophic algae can survive several days without light, a prolonged delay in shipping time may be fatal, as may exposure to extreme temperatures.

Prompt treatment of organisms is required on receipt of the shipment. Specimens obtained for rearing should be placed on rearing media, whereas those obtained for DNA isolation should be immediately frozen at or below –20 °C.

2. *Fresh plant samples*. Fresh plant tissue may be transported in two manners. For short transport duration (2–3 days, e.g., when acquiring samples from botanical gardens), leaf tissue or shoot cuttings in Ziploc bags can be carried at ambient temperature in insulated Styrofoam containers or mailed directly via overnight or express mail couriers, with or without Styrofoam containment.

A more reliable and expensive transport method involves placing the bags of tissue into an ice source inside a Styrofoam container. Wet ice may be used, but will melt and soak the package after a few days. Alternatively, a small amount of dry ice, wrapped in canvas, may be placed with the wet ice, to prevent the latter from melting. To avoid cold temperature burn, the Ziploc bags should be separated from the ice by means of newspaper.

Fresh tissue transport from remote locations requires phytosanitary, export and import permits. Tissue may be carried on wet ice in Styrofoam containers as airline hold or carry-on baggage [2]. Small packages require specific documentation to pass customs and agricultural inspection points in both shipping and receiving countries. Using airlines to ship packages is ill-advised, since many airlines refuse to handle a package originating abroad unless it has cleared customs, thus requiring a contact person. A copy of the USDA permit, phytosanitary certificate and letter identifying and describing the purpose of the plant tissue should be attached inside the package and outside with the shipping label (see below).

3. *Frozen samples.* Vertebrate tissue samples (e.g., blood and muscle) are routinely transported frozen. Transport of frozen tissue on dry ice or in liquid nitrogen is simple when air shipment is not required. However, authorisation for shipment of these chemicals on airlines is mandatory, since both are classified as “Dangerous Goods” [31]. Policies for packing, labelling and carrying such materials have been promulgated by the International Air Transport Association (IATA) in their *Dangerous Goods Regulations* manual, and general guidelines are provided on their website

(Appendix 2). Several courier services have instituted similar policies for the transportation of dangerous goods, e.g. the FX 12 Operator Variation of Federal Express.

Airlines permit no more than 200 kg of dry ice in a single container [3, 4]. The "Shipper's Declaration of Dangerous Goods" is required and the package must be marked as Hazard Class 9 (solid carbon dioxide). Handwritten declarations are not accepted; forms must be typewritten or computer-generated (refer to Appendix 1 for the website of Saf-T-Pak, which provides free online software for computer-generated Shipper's Declaration forms). Since cargo facilities may require pound equivalents, both metric and English equivalents should be used for packages. The special black-and-white sticker is a prerequisite for acceptance as air cargo.

Airlines permit up to 50 kg of liquid nitrogen to be transported in a nonpressurised Dewar container, which must also be marked as hazardous (compressed gas). The Shipper's Declaration is again required and the package must be clearly labelled "NONPRESSURISED LIQUID NITROGEN", "THIS SIDE UP" and "DO NOT DROP – HANDLE WITH CARE" to prevent careless handling and spillage. It may also require a green label stating "NONFLAMMABLE GAS".

Alternatively, the liquid nitrogen can be discarded before short flights and "Dry Shipper" (e.g., the Arctic Express Thermolyne CY 50915/50905 available from VWR Scientific Products, San Francisco, CA) used, thereby avoiding liquid nitrogen spillage. This is preferable for cryogenic collections because of the ease with which samples can be transported by air.

Sperm can be shipped on wet ice if it is collected undiluted in water and sodium azide has been added to a final concentration of 0.01–0.02%, in order to prevent bacterial growth [14]. For further guidelines on the transportation of frozen tissues, consult sections E1565 and E1566 in Volume 11.05 of the *Annual Book of ASTM Standards* of the American Society for Testing and Materials (West Conshohocken, PA) (Appendix 1).

4. *Desiccated samples.* Cultures of some fungi are stored lyophilised and can be shipped immediately on order [93]. Dried samples of skin, hair, feathers, muscle and blood from vertebrates may be placed in tubes or envelopes (in the case of blood spotted on paper) and mailed in regular mail [45]. Dried insects and dried plant tissues can be similarly transported: individual samples should be carefully wrapped in paper towel to prevent damage and placed inside vials or Ziploc bags. A small amount of desiccant (e.g., silica gel) should be included in the vials or bags. In the case of plant samples, this should be in contact with the leaf tissue. Sabrosky [152] and Piegler [153] provide guidelines for the packing and shipping of pinned insects.

Desiccated samples must be shipped in airtight containers to prevent rehydration and indicator silica gel is recommended to verify that the samples have not rehydrated [2].

5. *Fluid-preserved samples.* The most convenient method for transporting tissue samples of most invertebrates, vertebrates and marine algae, is preserved in 70–100% ethanol. However, since ethanol is flammable, highly volatile and a strong solvent (especially at higher concentrations), the use of unbreakable, leak-proof and ethanol-resistant plastic vials is a prerequisite to avoid evaporation or spillage. Sealing the vials inside thick, ethanol-resistant plastic sleeves is advised as a further precaution. Similar precautions against leakage should be ensured when transporting samples stored in other fluids. For further details, refer to McCoy [154].

Protocol 5 Packing and labelling for transportation

1. *Packing.* Sturdy, internally padded packaging should be used when shipping specimens or tissue samples. All external labels should be clearly marked with permanent ink and covered with a plastic sheet. A list of materials included, with relevant names, addresses and telephone numbers should be enclosed for the benefit of customs officials and to help recipients determine if the package was tampered with.

2. *Labelling.* When pertinent, the outside of the package may be marked "BIOMEDICAL SAMPLES FOR SCIENTIFIC RESEARCH – PLEASE RUSH", though for most packages, the words "SCIENTIFIC SPECIMENS FOR RESEARCH" will suffice. Customs forms should clearly indicate that the contents have "NO COMMERCIAL VALUE".

3. *Shipping.* Before shipping, ensure that holidays (civic and religious) will not impede or delay the delivery of packages. It is also prudent to avoid shipping over the weekend. These considerations are especially critical for shipments of live or frozen samples. Parcels of live samples should not be sent during periods of extremely hot or cold weather. Recipients should be called, faxed or contacted via electronic mail on the date of dispatch and notified of the expected arrival of the package and the airbill or tracking number. Recipients should be informed as to whether they can expect delivery or should collect their samples, and are advised to bring multiple copies of any permits or documentation necessary for collection. If transporting across international boundaries, colleagues on both sides should be intimately involved in the process of shipment.

5.2 Long-term Storage

5.2.1 Frozen tissues

Long-term storage conditions should minimise variation in temperature, light and liquid volume. Therefore, most formal frozen tissue repositories maintain samples at –130–150 °C in ultracold freezers or in the vapour phase of liquid nitrogen [155, 156]. Nonetheless, most plant, fungal and animal tissues will remain indefinitely stable for extraction of nucleic acids and proteins if maintained at –70–80 °C. Furthermore, although long term storage at or below –80 °C is the preferred method for animal samples (e.g., [65]), there are alternatives (Protocol 6).

For short-term storage, frozen algal samples can be kept in a standard freezer at –20 °C, in a mixture of glycol and dry ice (~ –78 °C) or in liquid nitrogen (–196 °C). For long-term storage, algal viability is more likely to be maintained at or below –40 °C. Routine, periodic monitoring of viability is essential for long-term storage of such samples.

Plant tissue samples have been stored at –20 °C, after quick freezing at –70 °C, with apparently no DNA degradation after 6–8 months [2]. However, marked degradation has also been observed when leaf tissue was stored at –20 °C, compared with –70 °C (no difference in DNA quality has been detected between tissue quick frozen in liquid nitrogen before being placed in the ultracold freezer relative to tissue simply placed in the freezer).

Storage at lower temperature is thus recommended and is essential if the tissue is to be used for isozyme analysis. Storage of tissues for RNA preparations, which require fresh or –70 °C frozen tissues, similarly necessitates cryogenic conditions for successful nuclease inhibition. When tissues are stored for such purposes, –70 °C freezers should be equipped with alarms and backup generators and temperatures should be checked on a regular basis, especially after storms or building maintenance [97]. Protocol 6 discusses additional considerations for the storage of frozen tissues.

If facilities for long-term storage of frozen samples are unavailable, investigators should consider depositing their samples in established frozen tissue collections upon completion of projects, for which guidelines for submission of samples are provided below. Further advice on cryopreservation, sources of the chemicals and the organisation of frozen tissue collections is provided by Dessauer et al. [3, 4], Simione [30], Baker and Hafner [97] and sections E1342, E1564, E1565 and E1566 in Volume 11.05 of the *Annual Book of ASTM Standards* (Appendix 1).

5.2.2 DNA

An alternative approach to tissue storage is to minimise or even eliminate storage in ultracold freezers and store only the DNA permanently (Protocol 6). Tissues are placed in the refrigerator immediately and DNA isolated as soon as possible. This is the preferred method in studies where fast DNA extraction methods are available, tissue can be processed rapidly and no backup tissue source for additional extractions of DNA or proteins is required.

There are several benefits of this approach: 1) the need for ultracold storage space is diminished, thus lowering the costs of purchasing equipment, electrical power and maintenance 2) space is made available for more important tissues where a backup tissue source is required 3) DNA samples are quickly and efficiently obtained 4) breakdown of ultracold freezers is less serious since DNA samples (which are more stable than tissue samples) have already been obtained.

Protocol 6 Common long-term storage methods

1. *Frozen samples.* Frozen tissue may be stored in an ultracold freezer, on dry ice or in liquid nitrogen tanks. Chest-type freezers use interior space more efficiently and maintain colder temperatures than upright models, although the latter require less floor space [28]. Permanent marker eventually deteriorates at extreme cold temperature, as do many kinds of tape, so placing a pencil label inside the sample holder is advised. Plastic tubes are preferable to glass for frozen storage, since sudden temperature changes may crack glass when samples are removed from the freezer.

The use of a cryoprotectant is advocated for frozen storage of many samples. For example, freezing and storage with a cryoprotectant has been advocated for preserving filamentous fungi in liquid nitrogen [157, 158], while algae can be stored as frozen samples with cryoprotectants such as glycerol, DMSO or dried milk solids [93].

2. *Desiccated samples.* Insect samples, plant material, feathers and hair may be stored dry at ambient temperature in the presence of a desiccant (e.g., silica gel). Spore-producing fungi have also been stored at ambient temperatures, usually for shorter periods of time, after drying in soil or silica gel. However, ambient temperatures cannot guarantee against DNA degradation in dry samples, especially if rehydration occurs [45]. In general, dry samples are a poor substitute for

fresh, frozen or ethanol-preserved samples in molecular studies and should be placed in ultracold storage on receipt.

3. *Fluid-preserved samples.* DNA yield and quality from specimens preserved in 95–100% ethanol are usually almost as good as from fresh or frozen tissue. Samples can be stored at ambient temperature, but long-term storage of samples in ethanol must be conducted under fireproof conditions. Storage of ethanol samples at –20 °C or colder is the best safeguard against long-term DNA degradation, since DNA in ethanol-preserved samples has been found to degrade after ca. six weeks at ambient temperature [65].

Samples should be stored in sealed vials (to prevent evaporation) and the ethanol should be periodically replaced. Glass vials are recommended since ethanol may weaken, and ultimately crack, certain plastics. For detailed discussions of the containers required for long-term storage of fluid-preserved samples, consult Palmer [159], Legler [160] and De Moor [161]. Hoebeke [162] described a unit-storage system for fluid-preserved samples.

Vertebrate tissue samples (e.g., blood) in 2% 2-phenoxyethanol (v/v), with glycerol or DMSO, are normally stored at –20 °C [45, 73]. However, Asahida et al. [79] developed a high concentration TNES-urea buffer suitable for preserving fish muscle and liver samples at ambient temperature for up to three years. Sperm containing 0.01–0.02% sodium azide can be stored refrigerated (5 °C) for 1–2 years, but must not be frozen [14].

4. *Fungal and algal cultures.* Fungal and algal samples may be stored as cultures, the long-term viability of which depends on species or strain, age of culture at time of storage, propagules present, culture medium (affecting nutritional state) and storage method. Extensive discussions of these techniques can be found in various reviews [105, 158, 163, 164]. Fungal stock cultures remain viable for at least 6 months on agar slants refrigerated at 5 °C. However, cultures in vials (15–20 ml) remain viable for longer periods. Most fungi survive at ambient temperature for 2–4 weeks. Wrapping cultures in parafilm retards desiccation of the medium, thus increasing the length of viability; contamination is also lessened. Cultures on agar may be covered with sterile mineral oil, to lower oxygen levels, or kept in sterile distilled water at 4 °C.

Some auxotrophic algae are quite similar to fungi in short-term storage requirements. However, most are phototrophic or photoauxotrophic and require adequate lighting. Temperatures slightly below ambient (~ 21 °C) and dim lighting are best for culture storage in liquid medium or agar. Some algae require special conditions (e.g., bubbling with air or CO_2), even for short-term

storage periods of 2–4 weeks. Detailed information on standard practices is contained in Stein [165].

Many fungal and algal cultures are viable after lyophilisation [93]. Lyophilisation of cultures has the advantage of preserving the genetic integrity of strains that might change during years of active growth.

5. *DNA*. Prepared DNA is best stored at 5 °C or in ethanol at –20 °C. High salt (> 1 M) in the presence of Na_2EDTA at pH 8.5 (Tris) buffer is recommended for long-term storage, along with storage in light-protected CsCl at 5 °C. High molecular weight DNA is stable at 20 °C for several days in buffer [1 mM Tris, 0.1 mM EDTA] and can be transported unrefrigerated.

5.3 Identification, Documentation and Storage of Voucher Specimens

5.3.1 Specimen identification

Organisms from which molecular data are obtained should be properly identified and documented. This is important for systematics and evolutionary studies because: 1) knowledge of the species involved may be crucial to interpretation 2) published molecular data may be utilised in ways other than for which they were initially obtained and 3) verifiability is one of the tenets of empirical science [14]. Given the effort required to obtain molecular data, it is the duty of the researcher to devote some care to proper identification and documentation.

If exemplar taxa are required as representatives of higher taxa, most groups contain common, well-known species that may be recognised by the nonspecialist with some confidence. However, the most reliable method of identification is to allow a specialist to provide identified material for molecular extraction, to identify specimens intended for use, or to identify voucher specimens from the same population. In addition to species identification, specialists can provide important information about taxon selection, unresolved relationships and model systems for studying comparative biological questions.

Publishing specialists can be located from the taxon indices of primary or reference journals like *Biological Abstracts*, specialist publications such as the *International Mycological Directory* [166] or *The Insect and Spider Collections of the World* [167], and the directories of societies, such as the *Directory and Guide of Resource Persons* of the American Society of Plant Taxonomists, *Resources in Entomology*, published by the Entomological Society of America, or the *Annuaire des Arachnologists Mondiaux* of the International Society of Arachnology. Several

directories, e.g., *Index Herbariorum*, are now available online (Appendix 8). Unfortunately, although they are generally willing to help with identification, specialists are often backlogged with work and may be unable to assist in a timely fashion. The task of identification then rests with the investigator, who has an array of literature, varying from pictorial identification guides to primary taxonomic papers, from which to choose.

Pictorial guides (e.g., [168]), written for the layman, are easiest to use, but are least inclusive and reliable. Colour photographs or line drawings are accompanied by short descriptions and distributional range of the most common species occurring in the region. Where several closely related species occur in the region, usually only one is included. Moreover, these guides avoid using morphological characters necessary for correct identification, which is limiting given the range of ontogenetic and geographical variation found among conspecifics. Field guides are usually more detailed, providing keys in addition to descriptions and figures (e.g., [169, 170]), but only a few allow identification below the level of order or family (e.g., [171]). For such information, the investigator must consult monographic identification manuals, containing detailed keys written by specialists. These are superior for identifying the better known species in the region they cover, but require proficiency in morphological terminology. Monographic series, e.g., the *Synopses of the British Fauna* (*New Series*) published by the Linnean Society, containing keys and numerous illustrations, are also available for the identification of selected groups. Although regional in coverage, such monographs are often useful over a wider range than suggested by their titles (e.g., [172]).

Primary taxonomic literature, scattered in books, monographs and journals, forms the basis for all guides and manuals. If this is familiar to the investigator, then it is the best place to start. Monographs (e.g., taxonomic catalogues or regional floras and faunas) are an essential introduction if the investigator is unfamiliar with the literature. For example, Sims [173] referenced primary literature for invertebrates worldwide by taxonomic group and region. Additional access to primary literature on particular groups can be obtained from the bibliographies of identification manuals and catalogues.

Ease of identification varies with taxonomic group and region. Some groups (e.g., butterflies and birds) are better known, as are some regions (e.g., north temperate). For many groups and as many regions, comprehensive identification manuals do not exist at all. In these situations, museums and herbaria (which usually maintain extensive reference libraries and microscope facilities) should be accessed for identifying organisms.

5.3.2 Documentation and voucher specimens

Due to the dynamic nature of systematics and the nomenclatural changes which accompany the discovery of new species or the reanalysis of phylogenetic relationships, organisms from which molecular data are published should be documented in such a manner that their identity can be verified should question arise. An essential aspect of this documentation is the voucher specimen(s), which should originate from the same local population (deme) as the experimental animals, and be placed into a permanent depository (museum or herbarium). Huber [174] defined voucher specimens in the broad sense as "all biological specimens having the minimum information of collection locality (ideally specified by latitude, longitude, altitude) and date that are preserved to document biological research."

Molecular systematists should not be naïve about the importance of vouchers and the necessity for retaining information about the organisms from which they have sampled molecules. Voucher specimens "physically and permanently document data in an archival report by 1) verifying the identity of the organism(s) used in the study and 2) by so doing, ensure that a study which otherwise could not be repeated can be accurately reviewed or reassessed" [94]. Vouchers serve as a backup and act as important documentation for ongoing systematics investigations [45, 175].

Exactly what comprises a voucher specimen and how many such specimens should be collected depends upon the taxon in question, the characters required for its identification and the number of specimens available [174]. For many taxa, diagnostic features of the specimen used as a tissue sample may suffice and may be all that is available if only a single specimen could be obtained for molecular analysis. However, it is preferable to retain a second, intact specimen as the voucher (if there is a choice between two specimens, a sexually mature individual should always be retained in preference and the immature specimen used as the tissue sample). Additional specimens, representing sexual and ontogenetic variation, should always be acquired, if possible. These may assist in the identification of sexually dimorphic taxa and may be invaluable in subsequent scoring of morphological data for a simultaneous cladistic analysis.

Photographs and sound recordings (e.g., of birds or anurans) should never be viewed as a replacement for vouchers, except in situations where endangered or threatened species are involved and populations have already been vouchered, e.g., Hawaiian Lobeliaceae [2]. However, photographic slide collections of organisms, from which tissue samples have been obtained, provide an excellent backup in documentation.

Although vouchers are usually obtained with field-collected material, they are often neglected when the tissue is obtained via an intermediate source (stock center, commercial supply company, botanical or zoological garden, colleague, etc.). In some cases (e.g., botanical and zoological

gardens), collections are numbered by accession and the original voucher information (collector and number) can be traced in records maintained by the institution. Such information should be obtained and recorded. However, it is prudent to have a second voucher made at the same time as the tissue is collected, to clarify label switches or errors in collecting.

Publication of molecular data should include the locality where the organisms were collected, date of collection, name of collector, depository of the voucher specimens and their catalogue numbers (where available). Catalogue numbers allow access to further information (e.g., the name of the specialist who identified the specimens) and are invaluable for computer archiving as well as storage and retrieval of samples (see below). For further discussions on the importance of voucher specimens, the reader is referred to Lee et al. [94], Huber [174], Meester [175], Robinson [176] and Yates [177].

5.3.3 Storage of vouchers

In common with all specimens, long-term storage of vouchers requires proper labelling and preservation, including retention of the characters pertinent to identification [94]. Different taxonomic groups have different requirements for preservation as specimens. For example, insects, plants, fungi, corals, some sponges, echinoids, asteroids, the skins and bones of birds and mammals, and the shells of molluscs, are stored dry. Most soft-bodied arthropods, worms and marine invertebrates (including the soft parts of molluscs), reptiles and amphibians, marine algae, and fungi are stored in ethanol or formalin, though use of the latter is waning. Among fluid-preserved specimens, there are further group-specific requirements. For example organisms with calcareous tests, shells or bones, which dissolve in formalin, must be stored in buffered formalin or ethanol [178–180]. Soft-bodied aquatic invertebrates, which contract severely when placed directly in fixative, must be relaxed first [181]. Finally, many invertebrates and fungi require sectioning or removal of the genitalia (in arthropods) or radulae (in molluscs), which must be specially fixed and slide-mounted to be suitable as vouchers (e.g., [62, 182–185]).

It is beyond the scope of this chapter to elaborate on the myriad of storage techniques for museum and herbarium specimens (all of which apply to vouchers). Most taxonomic groups have literature specifically devoted to the topic, such as Mueller [186] or Lincoln and Sheals [187] for the preservation of marine invertebrates, Hall [188] or Wagstaffe and Fidler [189] for vertebrates and Savile [190], Ketchledge [191] or Smith [192] for botanical specimens. Harris [193] and Huber [174] provide general guidelines for preservation of the major animal phyla. The onus rests with the investigator to determine which methods are most appropriate for the group in question

by consulting general texts on the preservation and curation of natural history collections (e.g., [94, 194–196]) and periodicals such as *Curator*, *Collection Forum* and the *Journal of Biological Curation*.

5.4 Archiving: Integrating Tissue Samples, Voucher Specimens and Collection Data into a Database

5.4.1 Practical considerations

Keeping track of tissue samples in the laboratory or in ultracold storage, of DNA (or protein) extracted from those samples, and of associated voucher specimens, is an important task when numerous concurrent molecular studies, involving many different investigators, are underway. Some laboratories maintain a decentralised system where each investigator keeps a separate record (e.g., in a spreadsheet) of their own samples. Others are fully centralised, all incoming tissues (and subsequently isolated DNAs) being assigned numbers upon arrival, from which all relevant information can be accessed in the database [156]. In either case, it is critical to include, or have referenced, all voucher information (collection locality, date of collection, collector, number of specimens, and depository). Additional information, e.g., quantity of tissue remaining, method of DNA extraction, date of extraction, quantity and quality of DNA, can also be recorded in the database [2].

Locating the frozen tissue or DNA sample listed in the database similarly requires appropriate organisation of freezers [97, 156]. Ultracold storage space is very expensive to purchase and maintain, so it is important that materials be stored in a space-efficient manner. It is also imperative that the access and inventory procedures for frozen tissue collections be extremely well organised. Separate boxes for holding frozen tissue samples can be maintained for each project or for related taxa, or can merely be assigned numbers according to date of acquisition. Cryoboxes (e.g., Nunc, Taylor-Wharton, Revco) are recommended for storing the samples in ultracold freezers or liquid nitrogen tanks (cryovats) and for storing DNAs in –20 °C freezers. These boxes may be further partitioned internally, to hold up to 100 1.5–2.0 ml cryogenic microcentrifruge tubes, if required. Metal (Revco) racks, designed to hold several such boxes, are suitable for additional organisation. The contents of both the ultracold storage boxes and the DNA storage boxes should be clearly marked to permit rapid entry and exit from the freezers or cryovats. For further discussion of the logistical aspects of long-term storage, consult Baker and Hafner [97].

5.4.2 Depositing samples into frozen tissue collections

As with the deposition of voucher specimens in a museum or herbarium collection, the deposition of tissue samples in a frozen tissue collection requires accurate documentation of the collection data pertinent to the sample and inclusion of those data with the sample [94, 97]. A record of what the sample was used for, and where the voucher specimens are deposited, should also be included. Specific institutions have their own requirements for deposition of samples in their collections and the investigator is advised to consult the latest information on their websites (Appendix 5) for further guidelines. If multiple samples are to be deposited, it is advisable to provide the data in machine-readable format or submit it electronically.

5.4.3 Computer databases

Irrespective of whether tissue samples and voucher specimens are deposited in the same laboratory, in separate collections within the same institution, or in separate institutions, a record of information pertinent to those samples and vouchers must be maintained in a computerized database. Numerous software packages are available to the investigator, varying from general database programs (e.g., Microsoft Access, Paradox) to programs designed specifically for the maintenance of biological collections (e.g., BIOTA, MUSE, Platypus, PRECIS). Woodward and Hlywka [197] developed a database strictly for managing frozen tissue collections and similar applications.

Ultracold freezers are very sensitive to even brief periods of temperature increase and every second that a freezer door is open while searching for a particular sample is energy-consuming and could eventually contribute to freezer failure [97]. Researchers must therefore know exactly where each sample is located before opening the freezer. Many of these problems can be minimised through the use of a computerized inventory system. Database programs specific to the organization of freezers include Freezerworks (DataWorks Development, Inc., Mountlake Terrace, WA), a commercial program which integrates a thermal transfer label printer and bar-code reader with the database software, and Frozen Cell Stock Monitor (FCSM), a virtual container program for individual workstations which is freely distributed by the authors [198].

Recently, there has been a drive to integrate collections maintenance software with software for other biological databases, such as cladistics, morphometrics, species description and virtual identification (e.g., DELTA, Specify, BIOLINK). Ultimately, the choice of software will depend

upon the needs of the investigator, the flexibility of the software (including its stability, potential for addition of new data, platform independence, and generality of datafile formats), the hardware requirements and the cost (some software, e.g., DELTA, DELTA Access and Specify are free). Further discussion on the computerization of collections can be found in McAllister et al. [199], Arnold [200], Wingate [201] and Owen [202], while the reader is directed to the websites listed in Appendix 9 for information on database software.

6 Legal and Ethical Issues

Last, but by no means least, legal and ethical considerations are an integral component of any research program involving the acquisition of specimens or tissue samples. These issues concern not only the manner in which samples are acquired – be it from the field, from commercial suppliers or from collections-based institutions – but where they may be deposited, and the publication of results obtained from their analysis. Throughout the course of a research program, researchers are legally obligated to abide by collection, exportation and importation regulations, and by the regulations of institutions concerning the loan and deposition of samples. In addition, researchers are ethically obligated to conduct their investigations with due respect to the organisms and the country of origin.

6.1 Permits to Collect, Export and Import Specimens and Tissue Samples

6.1.1 International collecting regulations

Genetic resources were once treated as a common heritage, available without restriction for research and other usage, but this viewpoint was perceived as unfair to developing countries – the major source of genetic resources [203, 204]. Since the 1992 Convention on Biological Diversity declared that governments have the "sovereign right to exploit" the genetic resources under their domain, efforts to regulate access have commenced. Permits regulating where collecting is conducted, what is collected, and how specimens or tissue samples are transported, are now mandatory in many countries, and may present the greatest hurdle (besides financial support) to collecting in developing nations.

Regulations governing wildlife collection and transportation are complicated. Policy directives are not always clear and are generally covered by multiple administrative branches. Since these institutions are often autonomous and may be unaware of each other's requirements or

enforcement efforts, there is no "one-stop shopping" for permits. For example, an expedition to the tepuis of Amazonas Territory, Venezuela, required seven different permits (from the Ministry of Environment, the Institute of National Parks, the Indian Affairs Bureau, the National Guard and the Governor of Amazonas Territory), more than one year's advance application, and a week of negotiations after arrival [2]. The profusion of regulations that apply at state or international level is beyond the scope of this chapter. However, some general recommendations are provided below.

Collecting permits typically require at least 6 months' advance application, but a year or more may be necessary for developing countries in Africa, Asia or Latin America. The regulations of countries may differ radically – e.g., Bolivia has no application process, whereas the processes in Mexico and Peru are lengthy and complex [2, 205] – and are subject to change without notice. Most permits require submission of a detailed proposal, an equally detailed report on completion of field work and reprints of publications ensuing from the work. In addition, permits usually require that some or all of the specimens collected (including any holotypes) be deposited in the national or local collection. Special permission may therefore be necessary to export unicate samples. Occasionally, a preliminary field trip report and/or a complete copy of the field notes must also be provided before the investigator is permitted to depart the country. Permits granted for an extended period of time (e.g., 6 or 12 months) may be renewable, but usually require periodic submission of progress reports or collaboration with local researchers [205].

Regulatory offices in many countries have neither time nor resources to respond to correspondence regarding permits from foreign nationals, hence it is advisable to first make contact with researchers in the host countries and with their consulates. Additional sources of information include societies such as the Association of Systematics Collections (Washington, D.C.), institutions that maintain staff or projects in the countries of interest (e.g., American Museum of Natural History, Smithsonian Institution, New York Botanical Garden) and fellow scientists who have recently travelled to such countries (Appendix 8). A professional venue for permits information is currently hosted by the Smithsonian Insitution (the listowner is Sally Shelton, Collections Officer of the National Museum of Natural History). This is a moderated cross-disciplinary listserver, intended to facilitate discussion and information flow on all issues related to the rapidly changing terrain of biological collecting, permits, access and import/export regulations. Refer to Appendix 2 for details on how to subscribe to the permits listserver as well as other relevant websites, e.g., the *Journal of International Wildlife Law & Policy* and the Wildlife Interest Group of the American Society of International Law, which include research

bibliographies on legal issues and links to the full text of national and regional wildlife legislation in many countries.

6.1.2 Threatened or endangered species

Special restrictions apply to the collection and transportation of threatened or endangered species, for which the sampling of tissue and the preparation of DNA bear legal responsibilities akin to the collecting of whole specimens [45]. Researchers should familiarise themselves with the regulations before applying for permits. Detailed information concerning personnel who will handle endangered species must be provided in permit applications, because heavy fines have been enforced for the violation of permit conditions [206]. Investigators are urged to include on their permit applications all students and technicians who perform field and laboratory protocols with materials from endangered species, and to promptly report any changes in protocols or personnel to the permit authority.

Researchers specialising in endangered taxa should also familiarise themselves with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permitting system. CITES is a comprehensive treaty signed by over 150 countries which regulates international trade to prevent the decline of species threatened (listed in Appendix I of CITES) or potentially threatened (listed in Appendix II) with extinction. “Trade” is defined as import, export or re-export of CITES-listed animal and plant specimens, regardless of whether or not commerce is involved. International shipment of endangered species listed in the CITES protocol is now strictly controlled in most countries and the importation into the U.S. (or any other participant nation) of any species (alive, dead or part) on the international endangered species list requires a permit. Trade is virtually prohibited in the case of Appendix I species and is restricted for Appendix II or III species. While there are many signatory nations to this treaty, each must pass its own enabling legislation, so that the implementation of CITES may vary from country to country. Because domestic authorities have no jurisdiction in foreign countries, they will seek to prosecute the importer of improperly shipped material. Accordingly, it is advisable to enlist the help of collaborators, fluent in the permitting process and in the languages on both sides of the border, when crossing international borders with CITES-regulated taxa. Similarly, researchers are advised to establish the reputation of individuals supplying tissues and to insist that they obtain the necessary permits to be included with the shipment of specimens. Otherwise, researchers may be guilty of receiving improperly packaged or transported materials.

Member countries and institutions of CITES can freely interchange materials with the proper permits. However, a CITES exemption or "Certificate of Scientific Exchange" possessed by many academic institutions does not replace the need for import, export or collecting permits. Such an exemption merely makes it possible for like institutions to conduct loan transactions without the need for management agency paperwork and there is no provision for individual collecting. For further information on endangered species, the researcher is referred to the websites of CITES and TRAFFIC, the wildlife trade monitoring program of the World Wildlife Fund (WWF) and the World Conservation Union (IUCN), provided in Appendix 2.

6.1.3 U.S. collecting regulations

State and federal agencies issue various permits regulating the collection, capture, holding and sampling procedures that pertain to plants and most wild or feral animals in the U.S.A. Where vertebrates are concerned, regulations may dictate the numbers and circumstances under which investigators may collect blood, hair, feathers, urine, saliva, semen, milk, eggs, venom, body tissues and whole carcasses, including road kills.

In contrast, few restrictions apply to invertebrates, unless they occur specifically on protected lands. However, the collection of certain freshwater or nearshore "shellfish" (edible molluscs, crustaceans and echinoderms) is controlled by state government and may require a collecting permit or fishing license. Investigators should determine beforehand whether the taxa of interest have any special restrictions or are unregulated, as in the case of designated pest species. The U.S. and international endangered species lists are published and updated annually in the U.S. Code of Federal Regulations, Title 50, parts 17 and 23 (see below). Copies of the lists and permit information may be obtained from the U.S. Fish and Wildlife Service (Appendix 2). Many states in the U.S. also have separate lists of endangered and threatened species for which permission to collect must be obtained from the state natural resource agency.

In general, few regulations pertain to collections on privately-owned land, unless threatened or endangered species are involved. However, permits are compulsory for collections made on protected federal lands, such as national parks, wildlife refuges, wilderness areas, conservancies, marine sanctuaries and monuments. Such permits are usually specific to species and quantity of material allowed for collection and expire within 6 or 12 months.

Similar regulations also apply to state parks and streams in most states. However, state regulations vary, depending on jurisdiction (fisheries *versus* land and natural resources offices). For example, all intertidal and marine organisms are protected in California and any collecting

conducted in the intertidal zone, or by scuba close to shore, requires a permit. General information regarding federal regulations can be obtained from the U.S. Fish and Wildlife Service, whereas information concerning regulations in a particular state must be acquired from the pertinent natural resource agency of the state. Inquiries concerning collecting in specific reserves should be addressed to the manager, superintendent or ranger in charge.

Researchers are urged to contact relevant agencies (Appendix 2) before planning experiments, especially if the investigation requires unstable or easily degraded materials, e.g., mRNAs of certain age or tissue specificity. When annual restrictions on take (e.g., sex, breeding *versus* nonbreeding season) apply to an organism, samples may only be obtainable at certain times, thereby influencing academic schedules and the starting dates of grants.

6.1.4 International transportation regulations

Many countries enforce strict regulations regarding the export of biological material, which may be more stringently controlled than regulations governing the collection of samples. For example, permission to collect specimens and tissue samples may only be granted on condition that some or all of the samples remain in the country of origin (as discussed above). The onus rests with the investigator to apply to the appropriate authorities in each country.

Import permits are similarly required when material reaches its destination. There are no prohibitions *per se* against the importation into the U.S. (or most other countries) of specimens or samples preserved in ethanol or formalin, or as extracted nucleic acids (insofar as these are all assumed to be sterile), unless they originate from an endangered species. However, customs officials may be unable to judge whether the samples are legal and may confiscate questionable material until that has been verified. In order to avoid such situations, the researcher should contact the appropriate agency beforehand. If a permit is not required, a letter to that effect should be obtained from the agency.

For some studies, it may be necessary to import fresh or live material (e.g., where organisms are small and must be cultured to obtain sufficient DNA, or if mechanistic or developmental studies are intended). Live plants and animals fall under the regulations of various agencies. For example, in Canada, live animal and animal product importation permits are issued by the Canadian Food Inspection Agency (CFIA), which maintains an Automated Import Reference System (AIRS) for internal use in monitoring permit requirements. In order to be issued with a permit by the CFIA, researchers must indicate the animal species, its country of origin and the reason for its importation (Appendix 2).

Procedures may stipulate how animals must be inspected and transported, in addition to the regular permits required for collecting, exporting and importing. For example, EU countries have strict regulations governing the humane transport of live animals, which include regulations stipulating the amount of time they are allowed to spend in transit (Appendix 2). These rules are based on guidelines published by the International Air Transport Association (IATA) in the IATA publication *Live Animals Regulations*.

Live animal importation permits usually require some testing certification or affidavit of disease-free status from the country of origin, in addition to their export permit. Similarly, a phytosanitary certificate is routinely required to demonstrate that plant material (fresh or desiccated) is pest-free before it can be exported. In general, African and Asian countries cannot certify their material, which therefore cannot be imported into EU countries or the U.S. Import permits may also require that the receiving laboratory and/or quarantine facility be government-inspected. Material of economically important plant groups (e.g., Rutaceae, Poaceae, Orchidaceae) are subject to especially stringent inspection for arthropods, viruses and fungal pathogens [2], while live animals of unknown status may require that a risk assessment be conducted prior to issuance of an importation permit. Finally, researchers are advised to ascertain whether state or provincial permits are required in addition to the federal or national regulations.

6.1.5 U.S. transportation regulations

Many different regulations govern the transportation of animals, plants or their parts into and out of the U.S.A. Lists of wildlife and plant species that specifically require a federal permit for importation include species that are endangered or threatened, protected by CITES, or deemed injurious, and include all migratory birds and marine mammals. Other restricted articles include, but are not limited to, the following: all sea turtle products; many reptile skins and leathers, especially those originating from South American countries; most wild bird feathers, mounted birds or bird skins; ivory from elephants, whales, walruses and narwhals; furs from most spotted cats and all marine mammals; corals; and many plant species including orchids, cacti and cycads. Federal regulations further prevent the importation of fish or wildlife into any state if that state's laws or regulations are more restrictive than any applicable federal treatment. Wild animals taken, killed, sold, possessed or exported to the U.S. in violation of any foreign laws are also denied entry. Applicable U.S. legislation is as follows:

1. *Endangered Species Act*: prohibits the import and export of species listed as endangered and most species listed as threatened. More than 1 000 species of animals and plants are officially

listed under U.S. law as endangered or threatened. Refer to *Endangered and Threatened Wildlife and Plants* (50 CFR 17.11 and 17.12) for annually updated lists of these taxa.

2. *Lacey Act*: prohibits the import, export, transport, sale, receipt, procurement or purchase in interstate or foreign commerce of animal species that have been taken, possessed, transported or sold in violation of any state or foreign law or taken or possessed in violation of other federal law or Indian tribal law.

3. *Marine Mammal Protection Act*: prohibits the import of marine mammals and their parts and products.

4. *Wild Bird Conservation Act*: regulates or prohibits the import of many exotic bird species.

The U.S. Department of Agriculture (USDA) permit is a prerequisite for entry of fresh plant or animal material into the U.S. from other countries, including Canada. Although the USDA permit can be obtained for specific taxa and investigators, it is prudent to include several investigators and an array of taxa in the application. Copies of the USDA import permit should be carried at all times and forwarded to colleagues who will be sending material from other countries, so that copies may be included inside the parcel and outside with the shipping label.

The USDA lists animals for which import permits are "not required." However, these may still require inspection upon arrival. Plants, cuttings, seeds, unprocessed plant parts and certain endangered species either require an import permit or are prohibited from entering the U.S. Endangered or threatened species of plants and plant products, if importation is not prohibited, will require an export permit from the country of origin. Every plant or plant part has to be declared to Customs and must be presented for inspection. Researchers planning to import fish, wildlife or any product or part thereof, are advised to check with the Customs or Fish and Wildlife Service in advance (Appendix 2).

If animals are to be shipped alive, state and federal regulations usually require quarantine, including agricultural inspections of cage litter for noxious weeds or invertebrates. Lengthy quarantine is also required when shipping animals between the continental U.S. and Hawaii or Alaska. Severe delays may likewise be expected when importing samples of public health, agricultural or veterinary importance (e.g., disease organisms, plant pathogenic fungi or insect pests) into the U.S., as the permit process requires official state and federal agriculture approval, which can take several months. Researchers should contact the Foreign Quarantine Program of the U.S. Public Health Service and the Animal and Plant Health Inspection Service (APHIS) of the USDA (Appendix 2). APHIS performs inspections, offers guidelines and handles import permits. All wildlife and wildlife products must enter or exit the U.S. at one of the following designated USDA inspection ports: Baltimore, MD; Boston, MA; Chicago, IL; Dallas/Ft. Worth,

TX; Honolulu, HI; Los Angeles, CA; Miami, FL; New Orleans, LA; New York, NY; Portland, OR; San Francisco, CA; Seattle, WA.

For a more detailed discussion of the regulations governing transport of specimens and tissue samples into the U.S., including examples of the official application forms, refer to Dessauer and Hafner [31]. Sheldon and Dittman [122] provide a discussion of the permitting procedures required for import and export of samples from U.S. frozen tissue collections.

6.2 Legal Issues Concerning Specimens and Tissue Samples in Collections

6.2.1 Depositing samples

Investigators wishing to deposit tissue samples in biorepositories must be prepared to sign an affidavit stating that the sample was collected in accordance with all applicable laws and regulations [207–209]. Collection files should contain copies of collecting permits issued to the original collector of the specimens and, if the material is imported into the U.S., should contain copies of the requisite USDA importation form [97, 205].

6.2.2 Loaning samples

Collections-based institutions vary in their policies regarding access to materials [210–212], especially when destructive sampling is involved [123]. Some are more flexible than others, depending on their experience with individual investigators and their familiarity with the proposed techniques. Researchers should understand that the dictates of the curator, who intends to conserve materials, are intellectually opposed to the ideals of the experimentalist, who intends to destroy them. Accordingly, investigators must realise that destructive sampling of specimens from some of these institutions is simply not feasible or, if it is, that rare specimens or taxa which do not have a plentiful representation in collections are unlikely to be made available. Rather than view the curator as a block to progress, the experimentalist should design laboratory protocols that maximise use of existing materials or sample precious materials in nondestructive ways, and avoid the use of exhibition-quality specimens when partial, but well-documented items will suffice.

Each institution has its own policies regarding access to, and amounts available from, any given specimen and these guidelines should be consulted, or the curators contacted, before loans are requested. Critical information on which loans are made may include the potential for

significant new knowledge, experimental design, skill of the researchers, site of proposed research, proposed quantity of tissue to be consumed, quantity of tissue available for loan, past or planned contribution to the collection, collaboration with contributors to the collection, etc. [97, 123].

Most institutions require that a researcher requesting tissues from a collection submit a proposal to the curator or collections manager, wherein the importance of the proposed work is outlined and the capability of the researcher to successfully use the loaned tissues is demonstrated. Graduate students may be required to submit supplementary materials from their thesis advisors, which include evidence that their advisor is conversant with the techniques involved (i.e., peer-reviewed publications using such methods). Almost all collections-based institutions also require that unused tissues and nucleic acids are returned to the source collection for storage and archiving after the research has been completed, and that molecular data are deposited in GenBank, EMBL or a comparable database. For a primer on policies concerning the acquisition and deacquisition of specimens and samples in natural history collections, refer to Hoagland [212].

6.3 Ethical Issues

6.3.1 Illegal samples

Legal regulations are intended to prohibit the wanton destruction of biodiversity for commercial profit. Unfortunately, these regulations are often difficult to enforce, especially where the commercial trade in plants and animals (or their products) is concerned [213]. Illegally collected plants and animals are constantly smuggled across international borders and may find their way into legally imported consignments from neighbouring countries. The researcher may be confronted with a rare sample, the source of which is dubious (e.g., alleged country of origin lies outside the species' known distributional range) and which may therefore be illegal. Although this may be impossible to determine (e.g., the dealer may supply false paperwork), the researcher must make a moral decision to reject the sample. It is unethical to deal with any institution or individual that does not abide by the regulations of state, national and international organisations.

6.3.2 Handling animals

Ethical issues also concern the treatment of vertebrates (and occasionally invertebrates) to be sampled for molecular studies. Most scientific institutions receiving federal grants carry internally constituted animal ethics advisory committees, which evaluate research proposals that include use of vertebrates. Permission to take small quantities of blood, urine, feathers, feces or fur from healthy animals is usually granted routinely, except in cases where investigators plan experiments that require animals to be housed on-site or that utilise protocols which can be expected to produce significant but unavoidable stress.

Besides specifying numbers to be sampled and times at which animals can be collected, regulatory agencies may dictate the preferred or permitted method of collecting for ethical reasons. In the case of passerine birds, the use of anesthesia for bleeding or even minor abdominal surgery, such as laparotomies, is not recommended [214]. The time permitted to hold animals for processing, while collecting bodily fluids and making morphological measurements, may be limited to one hour to minimise stress. Handling of birds, bats and animals with chemical defences requires special skills, hence close collaboration with a skilled field biologist, certified to handle such species, is advised in these cases. Further guidelines on methods of anesthesia are provided by Dessauer et al. [3, 4].

6.3.3 Research attitudes

A further ethical consideration when obtaining samples concerns the attitudes of researchers to regulations imposed by foreign countries. Many developing countries prohibit the export of any part (including isolated DNA and proteins or even PCR products that still contain some of the template DNA) of endangered or economically important species, which may encompass all biodiversity in some cases [205]. Although these regulations may be viewed as an obstacle to scientific progress and there may be technical ways of circumventing them (e.g., [215]), it is important to realise that such organisms are resources for the countries in question and should be respected as such [216].

Similarly, researchers and scientific administrators should become more aware of the ethical responsibility of providing credit to colleagues in developing countries for assistance rendered, which may include co-authorship of publications, acknowledgements and inclusion of foreign researchers on proposals to granting agencies [2, 97]. Many developing countries now encourage collaboration with local researchers for the promotion of permit applications [203]. For a primer on genetic resources policy, including discussions on national sovereignty, access to and

ownership of intellectual property rights, and the “common heritage of mankind”, refer to Stenson and Gray [204] and Hoagland and Rossman [217].

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Appendix

1 Molecular Biology Websites, Products and Services

- Molecular Biology Techniques Forum and Molecular Biology Resources, hosted by the Northwest Fisheries Science Center (NWFSC), National Marine Fisheries Service (NMFS), National Oceanographic and Atmospheric Administration (NOAA), US Department of Commerce, Seattle, WA, USA: http://www.nwfsc.noaa.gov/protocols/methods/methods.html; http://www.nwfsc.noaa.gov/protocols/resources.html
- Molecular Biology Databases on the Internet, hosted by the Weizmann Institute of Science, Rehovot, Israel: http://bioinfo.weizmann.ac.il/mb/db/species_specific_databases.html
- BioLinks, hosted by the Department of Molecular and Cell Biology (MCB), Harvard University, Cambridge, MA, USA: http://mcb.harvard.edu/BioLinks.html
- *Ancient DNA Newsletter*, The Zoological Society of London, Institute of Zoology, London, UK: http://www.londonzoo.co.uk/ioz
- *BioTechniques*, Eaton Publishing Co., Natick, MA, USA: http://www.biotechniques.com
- BioSupplyNet, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA: http://www.biosupplynet.com/index.cfm
- Perkin-Elmer, Inc., Wellesley, MA, USA: http://www.perkinelmer.com
- Kimberly-Clark Corporation, Dallas, TX, USA: http://www.kimberly-clark.com
- VWR Scientific Products, San Francisco, CA, USA: http://vwrsp.com
- Nalge-Nunc International Corporation, Rochester, NY, USA: http://www.nalgenunc.com
- Revco Technologies, Inc., Asheville, NC, USA: http://www.revcotech.com
- Taylor-Wharton International, Theodore, AL, USA: http://www.taylor-wharton.com
- For shipping products and services: Saf-T-Pak Inc., Edmonton, AB, Canada: http://www.saftpak.com
- To order sections E1342, E1564, E1565, E1566 of the *Annual Book of ASTM Standards* (Vol. 11.05), American Society for Testing and Materials (ASTM), West Conshohocken, PA, USA: E1342 (Practice for Preservation by Freezing, Freeze-Drying, and Low Temperature Maintenance of Bacteria, Fungi, Protista, Viruses, Genetic Elements, and Animal and Plant Tissues); E1564 (Guide for Design and Maintenance of Low-Temperature Storage Facilities for Maintaining Cryopreserved Biological Materials); E1565 (Guide for Inventory Control and Handling of Biological Material Maintained at Low Temperatures); E1566 (Guide for Handling Hazardous Biological Materials in Liquid Nitrogen): http://www.astm.org

2 Permits and Regulations

- The International Air Transport Association (IATA), Dangerous Goods Online: http://www.iata.org/cargo/dg
- To order IATA publications: *Dangerous Goods Regulations*: http://www.iata.org/ads/dgr.htm; *Live Animals Regulations*: http://www.iata.org/ads/lar.htm
- Import-export regulations for selected countries, hosted by the Biodiversity and Biological Collections Web Server: gopher://biodiversity.bio.uno.edu/11/curation/permits
- Treaty Database, American Society of International Law (ASIL), Wildlife Interest Group, Burlingame, CA, USA: http://www.eelink.net/~asilwildlife/docs.html
- *Journal of International Wildlife Law & Policy*, Kluwer Academic Publishers, Norwell, MA, USA: http://ww.jiwlp.com
- Permits for collecting and exporting samples from Australia: http://pioneer.mov.vic.gov.au/chaec/open.html
- Instituto Nacional de Biodiversidad (INBio), collecting permits for Costa Rica: http://www.inbio.ac.cr/en/default.html
- European Union environmental legislation: http://europa.eu.int/comm/environment/legis_en.htm
- Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), collecting permits for Mexico: http://www.conabio.gob.mx

- To obtain current listings of endangered and threatened species, and federal permits for the US: US Fish and Wildlife Service (FWS), International Affairs, Arlington, VA: http://international.fws.gov
- To find out about quarantine requirements in shipping to the US: US Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Hyattsville, MD: http://www.aphis.usda.gov; USDA APHIS import-export directory: http://www.aphis.usda.gov/oa/imexdir.html; USDA APHIS, Veterinary Services, National Center for Import and Export (NCIE): http://www.aphis.usda.gov/ncie; USDA, APHIS, Plant Protection and Quarantine (PPQ): http://www.aphis.usda.gov/ppq
- US Customs Service, Washington, DC, Importing and Exporting: http://www.customs.gov/impoexpo/impoexpo.htm
- To determine if the sample from a vertebrate is disease-free: National Wildlife Health Center, Madison, WI, USA: http://www.nwhc.usgs.gov
- To determine whether importation to the US requires special permits because of disease risks: Centers for Disease Control and Prevention (CDC), US Department of Health and Human Services, Atlanta, GA: http://www.cdc.gov
- Canadian Food Inspection Agency (CFIA), Nepean, ON, Canada: http://www.cfia-acia.agr.ca
- Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES): http://www.unep.ch/cites
- TRAFFIC, Cambridge, UK: http://www.traffic.org
- World Wildlife Fund (WWF), Washington, DC, USA: http://www.worldwildlife.org
- The World Conservation Union (IUCN), Gland, Switzerland: http://www.iucn.org
- To join the permits listserver: Email listserv@sivm.si.edu. No subject is required. In the body of the message, issue the command: Subscribe PERMIT-L Firstname Surname
- Examples of frozen tissue collections policy: http://abscweb.wr.usgs.gov/research/ammtap/request.htm; http://www.uaf.edu/museum/af/using.html; http://www.washington.edu/burkemuseum/tissuepolicy.html; http://www.lms.si.edu/tissuepolicy.html

3 Live Stock Centers and Seed Banks

- Database of Laboratory Animals in Japan, University of Tokushima, Tokushima, Japan: http://www.anex.med.tokushima-u.ac.jp/index.html
- *E. coli* Collection, National Institute of Genetics, Mishima, Japan: http://shigen.lab.nig.ac.jp/ecoli/strain
- *E. coli* Reference Collection (ECOR) and DEC *E. coli* Collection, Penn State University, University Park, PA, USA: http://www.bio.psu.edu/People/Faculty/Whittam/Lab/ecor; http://www.bio.psu.edu/People/Faculty/Whittam/Lab/deca
- *E. coli* Genetic Stock Center (CGSC), Yale University, New Haven, CT, USA: http://cgsc.biology.yale.edu
- *Salmonella* Genetic Stock Center (SGSC), University of Calgary, AB, Canada: http://www.ucalgary.ca/~kesander
- Soil Microbiology Division, International Rice Research Institute, Manila, Philippines: http://www.irri.org
- *Chlamydomonas* Genetics Center, Duke University, Durham, NC, USA: http://www.biology.duke.edu/chlamy
- *Pseudomonas* Genetics Stock Center (PGSC), University of East Carolina, Greenville, NC, USA: http://www.pseudomonas.med.ecu.edu/Pseudomonas/index1.html
- Microalgal Biotechnology Labs and Biomass Conversion/Organic Synthesis Labs, Center for Basic Sciences, National Renewable Energy Laboratory (NREL), Golden, CO, USA: http://www.nrel.gov/basic_sciences/biosci.html
- Fungal Genetics Stock Center (FGSC), University of Kansas Medical Center, Kansas City, KS, USA: http://www.fgsc.net
- US Department of Agriculture Agricultural Research Service (USDA-ARS), US Plant, Soil and Nutrition Laboratory, Ithaca, NY, USA: http://www.arserrc.gov/naa/home/fedpsnl.htm

- USDA Forest Service, Forest Products Laboratory (Center for Forest Mycology), Madison, WI, USA: http://www.fpl.fs.fed.us
- National Germplasm Resources Laboratory (NGRL), Beltsville, MD, USA, and National Germplasm Repositories of the USDA National Plant Germplasm System (NPGS): http://www.barc.usda.gov/psi/ngrl/ngrl.html; http://www.ars-grin.gov/npgs/rephomepgs.html; http://www.ars-grin.gov/npgs/holdings.html
- National Seed Storage Laboratory (NSSL), Fort Collins, CO, USA: http://www.ars-grin.gov/ars/NoPlains/FtCollins
- The *Arabidopsis* Information Resource (TAIR): http://www.arabidopsis.org
- SENDAI *Arabidopsis* Seed Stock Center (SASSC), Department of Biology, Miyagi University of Education, Miyagi, Japan: http://shigen.lab.nig.ac.jp/arabidopsis
- Nottingham *Arabidopsis* Stock Center (NASC), The University of Nottingham, Nottingham, UK: http://nasc.nott.ac.uk/home.html
- *Arabidopsis* Information Management System (AIMS), *Arabidopsis* Biological Resource Center, Columbus, OH, USA: http://aims.cse.msu.edu/aims
- Barley Germplasm Database, National Institute of Genetics, Mishima, Japan: http://www.shigen.nig.ac.jp/barley/Barley.html
- Maize Genetics Cooperation Stock Center, University of Illinois at Urbana, Champaign, IL, USA: http://w3.aces.uiuc.edu/maize-coop
- Maize Germplasm Collection, North Central Regional Plant Introduction Station, Iowa State University, Ames, IA, USA: http://w3.ag.uiuc.edu/maize-coop/ncrpis.html
- Oryzabase, Rice Genetic Resources Center, National Institute of Genetics, Mishima, Japan: http://shigen.lab.nig.ac.jp/rice/oryzabase
- US Potato Gene Bank, Sturgeon Bay, WI, USA: http://www.ars-grin.gov/ars/MidWest/NR6
- Tomato Genetic Resources Center (TGRC), University of California, Davis, CA, USA: http://tgrc.ucdavis.edu
- US National Arboretum, Woody Landscape Plant Germplasm Repository, Glenn Dale, MD, USA: http://www.ars-grin.gov/cgi-bin/npgs/html/site_holding.pl?NA
- The Millenium Seed Bank Project, Science and Horticulture, Royal Botanic Gardens Kew, Richmond, UK: http://www.rbgkew.org.uk/seedbank
- *Index Seminum*: Seed Exchange (selected websites): http://www.unimo.it/ortobot/seedexc/seedexc.htm; http://www.helsinki.fi/ml/botgard/indesem.html; http://www.toyen.uio.no/botanisk/bothage/catalog
- American Fern Society (AFS) Spore Exchange, Hacienda Hts., CA, USA: http://amerfernsoc.org/sporexyy.html
- Nematode Collection, University of California, Davis, CA, USA: http://ucdnema.ucdavis.edu/imagemap/nemmap/museum.htm
- *Caenorhabditis* Genetics Center (CGC), University of Minnesota, St. Paul, MN, USA: http://biosci.umn.edu/CGC/CGChomepage.htm
- *Drosophila* Genetic Resources, National Institute of Genetics, Mishima, Japan: http://www.shigen.nig.ac.jp/fly/nighayashi.html
- *Drosophila* Stock Center, Facultad de Ciencias, Universidad Nacional Autónoma (UNAM), México: http://hp.fciencias.unam.mx/Drosophila/LOSHTML/portada.html
- The *Drosophila* Species Stock Center, Tucson, AZ, USA: http://stockcenter.arl.arizona.edu
- *Drosophila melanogaster* Stock Center, Indiana University, Bloomington, IN, USA: http://flybase.bio.indiana.edu/stocks
- Szeged *Drosophila melanogaster* P Insertion Mutant Stock Center, Department of Genetics, Józef Attila University, Szeged, Hungary: http://gen.bio.u-szeged.hu/stock
- Exelixis Inc., EP Flystation, San Francisco, CA, USA: http://cdigraphics.com/flystation2
- Mosquito Colonies, Department of Medical Entomology, South African Institute for Medical Research (SAIMR), Johannesburg, South Africa: http://www.wits.ac.za/fac/med/entomology/resource.htm
- Instituto Butantan, São Paolo, Brazil: http://bernard.pitzer.edu/~lyamane/butantan.htm
- *Tribolium* Stock Center, Kansas State University, Manhattan, KS, USA: http://bru.usgmrl.ksu.edu/beeman%5Ctribolium

- Zebrafish Information Network (ZFIN): http://zfin.org
- Zebrafish International Resource Center (ZFIN), University of Oregon, Eugene, OR, USA: http://zfin.org/zf_info/stckctr/stckctr.html
- The Indiana University Axolotl Colony, Indiana University, Bloomington, IN, USA: http://www.indiana.edu/~axolotl
- Jax® Mice, The Jackson Laboratory, Bay Harbor, ME, USA: http://jaxmice.jax.org/index.shtml
- Mutant Mouse Database, Oak Ridge National Laboratory, Oak Ridge, TN, USA: http://lsd.ornl.gov/htmouse
- *Peromyscus* Genetic Stock Center, University of South Carolina, Columbia, SC, USA: http://stkctr.biol.sc.edu

4 Cell Lines and Culture Collections

- Common Access to Biological Resources and Information (CABRI) Consortium: http://www.cabri.org
- Shared Information of Genetic Resources (SHIGEN), National Institute of Genetics, Mishima, Japan: http://shigen.lab.nig.ac.jp
- Genetic Resource Databank (GRD), National Institute of Genetics, Mishima, Japan: http://www.shigen.nig.ac.jp/grd
- Cell Line Database (CLDB) and Interlab Project, Genova, Italy: http://www.biotech.ist.unige.it/interlab/cldb.html
- Interlab Cell Line Collection (ICLC), Genova, Italy: http://www.iclc.it
- Coriell Cell Repositories, National Institute of General Medical Sciences (NIGMS), National Institutes of Health, Bethesda, MD, USA: http://locus.umdnj.edu
- The University of Michigan Breast Cell/Tissue Bank and Data Base, University of Michigan, Ann Arbor, MI, USA: http://www.cancer.med.umich.edu/umbnkdb.html
- Japanese Collection of Research Bioresources (JCRB) Cell Bank and Human Science Research Resources Bank (HSRRB), National Institute of Health Sciences (NIHS), Tokyo, Japan: http://cellbank.nihs.go.jp
- The National Laboratory for the Genetics of Israeli Populations (NLGIP), Tel Aviv University, Tel Aviv, Israel: http://www.tau.ac.il/medicine/NLGIP/nlgip.htm
- American Type Culture Collection (ATCC), Manassas, VA, USA: http://www.atcc.org
- European Collection of Cell Cultures (ECACC), Center for Applied Microbiology and Research (CAMR), Salisbury, UK: http://www.biotech.ist.unige.it/cldb/descat5.html
- World Federation for Culture Collections-Microbial Resources Centers (WFCC-MIRCEN), World Data Centre for Microorganisms (WDCM), Wako, Saitama, Japan: http://wdcm.nig.ac.jp
- Home Pages of Culture Collections in the World, hosted by WFCC-MIRCEN: http://wdcm.nig.ac.jp/hpcc.html
- Microbial Strain Data Network (MSDN), Sheffield, UK: http://panizzi.shef.ac.uk/msdn
- UK National Culture Collections (UKNCC): http://www.ukncc.co.uk
- US Federation for Culture Collections (USFCC): http://methanogens.pdx.edu/usfcc
- Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), The German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany: http://www.dsmz.de/index.html
- The German Collection of Human and Animal Cell Cultures, The German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany: http://www.biotech.ist.unige.it/cldb/coll121.html
- The Netherlands Culture Collection of Bacteria (NCCB), formerly LMD and Phabagen, Utrecht University, Utrecht, The Netherlands: http://www.cbs.knaw.nl/nccb/database.htm
- The Culture Collection of Algae and Protozoa, Institute of Freshwater Ecology (IFE) Windermere Laboratory at Ambleside and Dunstaffnage Marine Laboratory (DML) at Oban, UK: http://www.ife.ac.uk/ccap
- The Culture Collection of Algae at The University of Texas Austin (UTEX), Austin, TX, USA: http://www.bio.utexas.edu/research/utex
- Culture Collection of Algae, Department of Cell Biology and Applied Botany, Philipps-Universität, Marburg, Germany: http://staff-www.uni-marburg.de/~cellbio/welcomeframe.html

- National Collection of Type Cultures and Pathogenic Fungi (NCTC), Public Health Laboratory Service (PHLS), UK: http://www.phls.co.uk/services/nctc/index.htm
- University of Alberta Microfungus Collection and Herbarium (UAMH), University of Alberta, Edmonton, AB, Canada: http://www.devonian.ualberta.ca/uamh
- Culture Collection of Microorganisms, Institute for Fermentation, Osaka (IFO), Japan: http://wwwsoc.nacsis.ac.jp/ifo/microorg/microorg.htm
- USDA Agricultural Research Service (ARS) Microbial Culture Collection (NRRL), Peoria, IL, USA: http://nrrl.ncaur.usda.gov
- Molecular Pathology Shared Resource, Cancer Center, University of California San Diego (UCSD), CA, USA: http://cancer.ucsd.edu/molpath/examples.htm
- Molecular Pathology Collection (frozen and archival human pancreatic ductal carcinomas, metastatic lesions, preneoplastic lesions, and tumor-derived cell lines), Allegheny University, Philadelphia, PA, USA: http://www.mcphu.edu/research/rspt/resdb/frozenandarchival.html
- The Cloning Vector Collection, National Institute of Genetics, Mishima, Japan: http://www.shigen.nig.ac.jp/cvector/cvector.html
- cDNA libraries available commercially: CLONTECH Laboratories, Inc., Palo Alto, CA, USA: http://www.clontech.com/index.shtml; OriGene Technologies, Inc., Rockville, MD, USA: http://www.origene.com/cdnalib.htm; Stratagene, La Jolla, CA, USA: http://www.stratagene.com/home.htm

5 Frozen Tissue Collections

- Departamento de Ciencias Biológias, Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina: http://www.uncu.edu.ar
- Department of Biological Sciences, Macquarie University, Sydney, NSW, Australia: http://www.bio.mq.edu.au
- School of Biological Science, University of New South Wales, Sydney, NSW, Australia: http://www.bioscience.unsw.edu.au
- School of Biological Sciences, University of New England, Armidale, NSW, Australia: http://www.une.edu.au/sciences/schoolbiosci/index.html
- CSIRO Marine Laboratories, Cleveland, QLD, Australia: http://www.bne.marine.csiro.au
- The Australian Biological Tissue Collection, Evolutionary Biology Unit, South Australia Museum, Adelaide, SA, Australia: http://www.samuseum.sa.gov.au/ebu.htm
- Institut für Medizinische Chemie, Veterinärmedizinische Universität Wien, Wien, Austria: http://www-med-chemie.vu-wien.ac.at
- Royal Ontario Museum and Department of Zoology, University of Toronto, ON, Canada: http://www.rom.on.ca/index.html; http://www.zoo.utoronto.ca
- Redpath Museum, McGill University, Montreal, QC, Canada: http://www.redpath-museum.mcgill.ca
- Finnish Game and Fisheries Research Institute, Ahvenjarvi Game Research Station, Ilomantsi, Finland: http://www.rktl.fi/english
- Section de Biologie, Institut Curie, Paris, France: http://www.curie.fr
- Institut des Sciences de l'Evolution, Université Montpellier II, Montpellier, France: http://www.isem.univ-montp2.fr
- Departement de Biologie-Ecologie, Université Paul Valéry, Université Montpellier III, Montpellier, France: http://alor.univ-montp3.fr/epe/Index.html
- Department of Zoology, Hessisches Landesmuseum Darmstadt (HLMD), Darmstadt, Germany: http://www.darmstadt.gmd.de/Museum/HLMD
- Biology Department, Philipps-Universität, Marburg, Germany: http://www.uni-marburg.de/biologie/welcomeengl.html
- Institut für Physiologie, Universität Regensburg, Regensburg, Germany: http://www.biologie.uni-regensburg.de/InstituteNWFIII/physiologie.html
- Max Planck Institute of Biochemistry, Martinsried, Germany: http://www.biochem.mpg.de/home_en.html

- The Institute of Evolution, University of Haifa, Haifa, Israel: http://research.haifa.ac.il/~evolut/~evolut.html
- Department of Chemistry, Faculty of Science and Graduate School of Science, Tohoku University, Aobayama, Sendai, Japan: http://www.chem.tohoku.ac.jp/index-e.html
- Department of Biology, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia: http://fsas.upm.edu.my/~arahim/jabbio/index.html
- Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia: http://biology.um.edu.my/home.html
- Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands: http://www-bioch.sci.kun.nl/bioch/ger.html
- Department of Clinical and Human Genetics, Faculty of Medicine, Free University, Amsterdam, The Netherlands: http://www.med.vu.nl/org/afd/kga
- Department of Human and Clinical Genetics, University of Leiden, Leiden, The Netherlands: http://www.medfac.leidenuniv.nl/humangenetics/stages_nl.html
- Department of Biology and Nature Conservation, Zoological Institute, Agricultural University of Norway, Ås, Norway: http://wwwnlh.nlh.no/institutt/ibn/default_english.htm
- Department of Anatomy, Faculty of Medicine, Jagiellonian University, Krakow, Poland: http://www.cm-uj.krakow.pl/guide/cm4133.html
- Institute of Zoology, Academia Sinica, Taipei, Taiwan, Republic of China: http://www.sinica.edu.tw/zool/english/eindex.htm
- Department of Anatomy and Biology, National Defense Medical Center, Taipei, Taiwan, Republic of China: http://www.ndmctsgh.edu.tw
- Laboratory of Isotopic Investigations, Engelhardt Institute of Molecular Biology, Moscow, Russia: http://www.imb.ac.ru
- Department of Genetics and Pathology, Uppsala University, Uppsala, Sweden: http://www.genpat.uu.se
- Zoological Institute, Department of Biology, University of Bern, Bern, Switzerland: http://www.cx.unibe.ch/zos/zoologie
- Institute of Ecology – Laboratory for Zoology, Université de Lausanne, Lausanne-Dorigny, Switzerland: http://www.unil.ch/izea
- Laboratory of Molecular Biology, Institute of Clinical Pathology, University Hospital, Zürich, Switzerland: http://www.unizh.ch/pathol
- Department of Biology, School of Environment and Life Sciences, University of Salford, Salford, UK: http://www.els.salford.ac.uk/proper/biology.htm
- Department of Human Sciences, Faculty of Science, Loughborough University, Leicestershire, UK: http://www.lboro.ac.uk/departments/hu/index.html
- School of Biology and Biochemistry, Queen's University, Belfast, Northern Ireland, UK: http://www.qub.ac.uk/bb
- National Marine Fisheries Service (NMFS) Laboratories, National Oceanographic and Atmospheric Administration (NOAA), US Department of Commerce, USA: http://www.websites.noaa.gov/guide/government/nmfs.html; http://www.nmfs.noaa.gov/regional.htm
- Department of Biological Sciences, University of Alabama, Tuscaloosa, AL, USA: http://www.as.ua.edu/biology/scf
- Alaska Frozen Tissue Collection, University of Alaska Museum, University of Alaska Fairbanks, Fairbanks, AK, USA: http://zorba.uafadm.alaska.edu/museum/af/index.html
- Alaska Department of Fish and Game, Gene Conservation Laboratory, Anchorage, AK, USA: http://www.cf.adfg.state.ak.us/geninfo/research/genetics/genetics.htm
- Auke Bay National Marine Fisheries Laboratory, National Marine Fisheries Service (NMFS), National Oceanographic and Atmospheric Administration (NOAA), US Department of Commerce, Juneau, AK, USA: http://www.afsc.noaa.gov/abl
- Alaska Marine Mammal Tissue Archival Project (AMMTAP), Anchorage, AK, USA: http://abscweb.wr.usgs.gov/research/ammtap/index.htm
- Department of Pharmacology, University of Arizona College of Medicine, Tucson, AZ, USA: http://www.pharmacology.arizona.edu/Pharm_Tox

- Laboratory of Molecular Systematics, California Academy of Sciences, San Francisco, CA, USA: http://www.calacademy.org/research
- Museum of Vertebrate Zoology (MVZ), University of California, Berkeley, CA, USA: http://www.mip.berkeley.edu/mvz/collections/TissueCollection.htm
- Center for Reproduction of Endangered Species (CRES), Zoological Society of San Diego, San Diego, CA, USA: http://sandiegozoo.org/cres/frozen_resourcelist.html
- Division of Biological Sciences, Section of Ecology and Evolution, University of California, Davis, CA, USA: http://www.eve.ucdavis.edu
- Beckman Research Institute, Duarte, CA, USA: http://bricoh.coh.org/docs
- Department of Organismic Biology, Ecology and Evolution, University of California, Los Angeles, CA, USA: http://www.obee.ucla.edu
- Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO, USA: http://www.bmb.colostate.edu
- Department of Biological Sciences, University of Connecticut, Storrs, CT, USA: http://www.biology.uconn.edu
- Peabody Museum of Natural History, Yale University, New Haven, CT, USA: http://www.peabody.yale.edu/collections
- Department of Biology, University of Miami, Coral Gables, FL, USA: http://fig.cox.miami.edu
- Department of Chemistry, Florida State University, Tallahassee, FL, USA: http://www.chem.fsu.edu
- Conservation Genetics Laboratory, Nova Southeastern University Oceanographic Center, Dania Beach, FL, USA: http://www.nova.edu/ocean
- Department of Biochemistry and Molecular Biology, University of Florida Medical School, Gainesville, FL, USA: http://www.med.ufl.edu/biochem
- Department of Small Animal Clinical Sciences, University of Florida College of Veterinary Medicine, Gainesville, FL, USA: http://www.vetmed.ufl.edu/sacs/index.htm
- Department of Zoology, University of Florida, Gainesville, FL, USA: http://www.zoo.ufl.edu
- Savannah River Ecology Laboratory, Institute of Ecology, Aiken, SC, USA: http://www.uga.edu/srel/DNA_Lab/dna_lab.htm
- School of Integrative Biology, University of Illinois, Urbana, IL, USA: http://www.life.uiuc.edu/sib
- Field Museum of Natural History, Chicago, IL, USA: http://www.fmnh.org/research_collections
- Organismal Biology and Anatomy, Division of Biological Sciences, University of Chicago, Chicago, IL, USA: http://pondside.uchicago.edu/oba
- Department of Biology, Indiana University, Bloomington, IN, USA: http://www.bio.indiana.edu
- Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, USA: http://www.iupui.edu/~micro
- Museum of Natural History, University of Kansas, Lawrence, KS, USA: http://www.nhm.ku.edu/research.html
- Collection of Genetic Resources, Museum of Natural Science, Louisiana State University, Baton Rouge, LA, USA: http://www.museum.lsu.edu/LSUMNS/Museum/NatSci/tissues.html
- Division of Biochemistry and Molecular Biology, Department of Biological Sciences, Louisiana State University, New Orleans, LA, USA: http://www.biology.lsu.edu/bmb
- Department of Biology, College of Life Sciences, University of Maryland, College Park, MD, USA: http://www.life.umd.edu/biology/index.html
- Laboratory of Genomic Diversity, Frederick Cancer Research and Development Center, National Cancer Institute, National Institutes of Health, Frederick, MD, USA: http://rex.nci.nih.gov/lgd/front_page.htm
- Captive Propagation Research Group, Patuxent Wildlife Research Center, U.S. Geological Survey (USGS) and U.S. Fish and Wildlife Service (FWS), Laurel, MD, USA: http://www.pwrc.usgs.gov; http://patuxent.fws.gov
- Department of Entomology, University of Massachusetts, Amherst, MA, USA: http://www.umass.edu/ent/index.html
- Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI, USA: http://www.med.wayne.edu/anatomy

- Department of Biology, University of Michigan, Ann Arbor, MI, USA: http://www.biology.lsa.umich.edu
- Bell Museum of Natural History, University of Minnesota, Minneapolis, MN, USA: http://www1.umn.edu/bellmuse/collections.html
- Division of Biological Sciences, University of Montana, Missoula, MT, USA: http://biology.umt.edu
- Center of Theoretical and Applied Genetics, Department of Ecology, Evolution and Natural Resources, Rutgers University, New Brunswick, NJ, USA: http://www.rci.rutgers.edu/%7Edeenr/index.html
- Rutgers University Cell and DNA Repository, Nelson Biological Laboratories, Rutgers University, Piscataway, NJ, USA: http://lifesci.rutgers.edu/~genetics
- Division of Biological Materials, Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM, USA: http://www.unm.edu/~museum
- Ambrose Monell Collection for Molecular and Microbial Research, American Museum of Natural History, New York, NY, USA: http://www.amnh.org/science/genomics/research/frozen.html
- Bassett Healthcare Research Institute, Cooperstown, NY, USA: http://www.bassetthealthcare.org/research/programs.html
- Department of Biological Sciences, Fordham University, Bronx, NY, USA: http://www.fordham.edu/biology
- Department of Biology, University of Rochester, Rochester, NY 14267, USA: http://www.rochester.edu/College/BIO
- Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA: http://www.es.cornell.edu
- Bronx Zoo and Wildlife Conservation Society, Bronx, NY, USA: http://wcs.org/home/science/genetics
- Department of Biology, King's College, Briarcliff Manor, NY, USA: http://www.kings.edu/biology/bioweb.html
- Roswell Park Memorial Institute, Buffalo, NY, USA: http://www.cog.ufl.edu/publ/inst/inpg4920.htm
- Biology Department, Belmont Abbey College, Belmont, NC, USA: http://www.belmontabbeycollege.edu
- Cincinnati Museum of Natural History, University of Cincinnati, Cincinnati, OH, USA: http://www.cincymuseum.org
- Forensic Laboratory, U.S. Fish and Wildlife Service, Ashland, OR, USA: http://www.lab.fws.gov
- Academy of Natural Sciences, Philadelphia, PA, USA: http://www.acnatsci.org
- Department of Psychiatry, University of Pittsburgh School of Medical, Pittsburgh, PA, USA: http://www.wpic.pitt.edu
- Carnegie Museum of Natural History, Pittsburgh, PA, USA: http://www.clpgh.org/cmnh
- Veterinary Department, Riverbanks Zoo and Botanical Garden, Columbia, SC, USA: http://www.riverbanks.org
- National Biomonitoring Specimen Bank (NBSB), National Institute of Standards and Technology (NIST), Chemical Science and Technology Laboratory, Charleston, SC, USA: http://www.nist.gov/public_affairs/gallery/specimen.htm
- National Marine Mammal Tissue Bank, National Marine Fisheries Service (NMFS) Marine Mammal Health and Stranding Response Program, Ft. Johnson National Oceanographic and Atmospheric Administration (NOAA) Facility, Charleston, SC, USA: http://www.nmfs.noaa.gov/prot_res/PR2/Health_and_Stranding_Response_Program/mmhsrp.html
- Division of Biology, University of Tennessee, Knoxville, TN, USA: http://www.bio.utk.edu
- Department of Biology, Natural History Collection, Angelo State University, San Angelo, TX, USA: http://www.angelo.edu/dept/biology/info/asnhc.htm
- Department of Biological Sciences, University of North Texas, Denton, TX, USA: http://www.ias.unt.edu
- Natural Science Research Laboratory, Museum of Texas Tech University, Lubbock, TX, USA: http://www.nsrl.ttu.edu/collecti.htm
- Texas Cooperative Wildlife Collection, Texas A&M University, College Station, TX, USA: http://wfscnet.tamu.edu/tcwc/tissues.htm
- Texas Memorial Museum and Section of Integrative Biology, University of Texas, Austin, TX, USA: http://www.tmm.utexas.edu/research/index.html; http://www.biosci.utexas.edu/IB

- Department of Biology and Biochemistry, University of Houston, Houston, TX, USA: http://www.bchs.uh.edu
- Department of Zoology, College of Biology and Agriculture, Brigham Young University, Provo, UT, USA: http://bioag.byu.edu/zoology
- Department of Biology, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT, USA: http://www.uvm.edu/~biology
- Burke Museum, University of Washington, Seattle, WA, USA: http://www.washington.edu/burkemuseum/tissuepolicy.html
- Northwest Fisheries Science Center (NWFSC), National Marine Fisheries Service (NMFS), National Oceanographic and Atmospheric Administration (NOAA), US Department of Commerce, Seattle, WA, USA: http://research.nwfsc.noaa.gov/nwfsc-homepage.html
- Washington Department of Fish and Wildlife, Olympia, WA, USA: http://www.wa.gov/wdfw
- Laboratory of Molecular Systematics, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA: http://www.lms.si.edu/frozencollections.html
- National Zoological Park, Smithsonian Institution, Washington, DC, USA: http://natzoo.si.edu
- Department of Biological Sciences, Marshall University, Huntington, WV, USA: http://www.marshall.edu/biology
- Department of Physiology, University of Wisconsin Medical School, Madison, WI, USA: http://www.physiology.wisc.edu
- International Crane Foundation, Baraboo, WI, USA: http://www.savingcranes.org
- Molecular Systematics Laboratory, Zoological Museum, University of Wisconsin, Madison, WI, USA: http://www.wisc.edu/zoology/museum/museum.html
- Department of Biological Sciences, University of Wisconsin-Parkside, Kenosha, WI, USA: http://www.uwp.edu/academic/biology/biologymenu/Index.htm

6 Commercial Supply Companies

- Berkshire Biological Supply Co., Westhampton, MA, USA: http://www.crocker.com/~berkbio
- Carolina Biological Supply Co., Burlington, NC, USA: http://www.carolina.com
- Fisher Scientific, Educational Materials Division, Chicago, IL, USA: https://www1.fishersci.com/main.jsp
- Fluker Farms, Inc., Port Allen, LA, USA: http://www.flukerfarms.com
- Glades Herp., Inc., Ft. Myers, FL, USA: http://www.gherp.com
- Gulf Specimen Marine Laboratories, Inc., Panacea, FL, USA: http://www.gulfspecimen.org
- Marine Biological Laboratory, Marine Resources Center, Woods Hole, MA, USA: http://zeus.mbl.edu/public/organisms/catalog.php3
- Pacific Biological Supply, Sherman Oaks, CA, USA: http://www.pacificbio.com
- Ward's Natural Science Establishment, Inc., Rochester, NY and Santa Fe Springs, CA, USA: http://www.wardsci.com

7 Botanical and Zoological Gardens

- Botanic Gardens Conservation International (BCGI), an International Clearing House Mechanism for Botanic Gardens of the World, Royal Botanic Gardens Kew, Richmond, UK: http://www.bgci.org.uk
- Internet Directory for Botany (IDB), University of Alberta, Edmonton, AB, Canada: http://www.botany.net/IDB
- Lists of Botanical Gardens and Arboreta: http://dir.yahoo.com/Science/Biology/Botany/Botanical_Gardens; http://dir.yahoo.com/Science/Biology/Botany/Botanical_Gardens/Arboretums; http://www.botany.net/IDB/subject/botgard.html
- CAUZ Directory, The Consortium of Aquariums, Universities and Zoos (CAUZ) for Worldwide Conservation: http://www.selu.com/bio/cauz

- American Zoo and Aquarium Association (AZA) Zoo and Aquarium Directory: http://www.aza.org/members/zoo
- WWW Virtual Library: Zoos, hosted by ZooNet: http://zoonet.home.mindspring.com/www_virtual_lib/zoos.html
- The Cyber ZooMobile, Zoo Links: http://www.primenet.com/~brendel/zoo.html
- The Good Zoo Guide Online: http://www.goodzoos.com
- ZooNet: http://www.zoonet.org
- ZooWeb: World Wide Link to Zoos and Aquariums: http://www.zooweb.net
- Lists of Aquariums and Aviaries: http://dir.yahoo.com/Science/Biology/Zoology/Zoos/Aquariums; http://dir.yahoo.com/Science/Biology/Zoology/Zoos/Aviaries
- Public butterfly gardens and zoos, hosted by The Butterfly Website: http://www.mgfx.com/butterfly/gardens/index.cfm
- Insect Zoos, Butterfly Gardens and Museums in North America, hosted by the Entomological Society of America: http://www.entsoc.org/education/insect_zoos.htm

8 Natural History Museums, Herbaria and Directories of Specialists

- Association of Systematics Collections (ASC), Washington, DC, USA: http://www.ascoll.org
- Society for the Preservation of Natural History Collections (SPNHC): http://www.spnhc.org
- The Systematics Association, The Natural History Museum, London, UK: http://www.systass.org
- The Biodiversity and Biological Collections Web Server, University of New Orleans, New Orleans, LA, USA: http://biodiversity.uno.edu
- General Systematic Research Tools and Resources for Systematics Research, University of Michigan Herbarium, Ann Arbor, MI, USA: http://www.herb.lsa.umich.edu/tool_dir.htm
- Center for Biosystematics, University of California, Davis, CA, USA: http://cbshome.ucdavis.edu/index.html
- Virtual Library Museums Pages: Directory of Online Museums: http://www.icom.org/vlmp
- UCMP lists of Natural History Collections, Societies and Organisations, hosted by the Museum of Paleontology, University of California (UCMP), Berkeley, CA, USA: http://www.ucmp.berkeley.edu/collections/other.html; http://www.ucmp.berkeley.edu/subway/nathistmus.html; http://www.ucmp.berkeley.edu/subway/nathistorg.html
- Directory of Research Systematics Collections (DRSC) of the National Biological Information Infrastructure (NBII), ASC and USGS, Washington, DC, USA: http://bp.cr.usgs.gov/drsc/drsc.cfm
- Natural History Museums and Collections: http://www.lib.washington.edu/sla/natmus.html; http://fsas.upm.edu.my/~arahim/jabbio/museum.html
- Biological Museums on the Web, hosted by The Biodiversity and Biological Collections Web Server, University of New Orleans, New Orleans, LA, USA: http://biodiversity.uno.edu/cgi-bin/hl?museum
- The Insect and Spider Collections of the World, hosted by Bernice Pauahi Bishop Museum, Honolulu, HI, USA: http://www.bishopmuseum.org/bishop/ento/codens-r-us.html
- Insects and Entomological Resources, hosted by Saint Anselm College, Manchester, NH, USA: http://www.anselm.edu/homepage/chieber/insects.html
- Iowa State Entomology Index: Insect Collections, Iowa State University, IA, USA: http://www.ent.iastate.edu/List/insect_collections.html
- Council of Heads of Australian Entomological Collections (CHAEC), hosted by Museum Victoria, Melborne, VIC, Australia: http://pioneer.mov.vic.gov.au/chaec
- International Society of Arachnology, National Museum of Natural History, Washington, DC, USA: http://www.ufsia.ac.be/Arachnology/Pages/ISA.html
- Polar Museums Directory, The Scott Polar Research Institute (SPRI), University of Cambridge, UK: http://www.spri.cam.ac.uk/lib/museums.htm
- Botanical Museums, Herbaria, Natural History Museums on the Internet Directory for Botany (IDB), University of Alberta, Edmonton, AB, Canada: http://www.botany.net/IDB/subject/botmus.html
- A list of herbaria, hosted by Tel Aviv Herbarium (TELA), Tel Aviv, Israel: http://www.tau.ac.il/lifesci/botany/herbaria.htm

- *Index Herbariorum* and *Plant Specialists Index*, Online Search Index for Institutions and People, hosted by The New York Botanical Garden, Bronx, NY, USA: http://www.nybg.org/bsci/ih/ih.html
- American Society of Plant Taxonomists (ASPT), Membership Directory: http://www.sysbot.org/members.htm
- Worldwide List of Internet Accessible Herbaria: http://www.ibiblio.org/botnet/flora/wwwlist2.html
- Further listings of herbaria: http://dir.yahoo.com/Science/Biology/Botany/Herbaria
- Mycologists Online, Worldwide Directory for Mycology and Lichenology, hosted by the Institute of Botany, Bratislava, Slovakia: http://web.savba.sk/botu/myco

9 Biodiversity and Biological Collections Database Software

- The Biodiversity and Biological Collections Web Server, University of New Orleans, New Orleans, LA, USA: http://biodiversity.uno.edu
- General Systematic Research Tools and Resources for Systematics Research, University of Michigan Herbarium, Ann Arbor, MI, USA: http://www.herb.lsa.umich.edu/tool_dir.htm
- Digital Taxonomy – An Information Resource on Biodiversity Data Management: http://www.geocities.com/RainForest/Vines/8695
- BIOLINK, CSIRO Publishing, Melbourne, VIC, Australia: http://www.biolink.csiro.au
- BIOTA: The Biodiversity Database Manager, Sinauer Associates, Inc., Sunderland, MA, USA: http://viceroy.eeb.uconn.edu/biota
- DELTA: http://biodiversity.bio.uno.edu/delta
- MUSE and Specify, Biodiversity Research Center, Lawrence, KS, USA: http://biodiversity.bio.uno.edu/muse; http://usobi.org/specify/Muse.html; http://www.usobi.org/specify
- Platypus, CSIRO Publishing, Melbourne, VIC, Australia: http://www.environment.gov.au/abrs/platypus.html
- Freezerworks, Dataworks Development, Inc., Mountlake Terrace, WA, USA: http://www.freezerworks.com

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4 Dessauer HC, Cole CJ, Hafner MS (1996) Collection and storage of tissues. In: DM Hillis, C Moritz, BK Mable (eds): *Molecular Systematics*. 2nd Edition. Sinauer, Sunderland, MA, 29–47

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Further reading

Dessauer, H. C. and Hafner, M. S. (1984). *Collections of Frozen Tissues: Value, Management, Field and Laboratory Procedures and Directory of Existing Collections*. Association of Systematics Collections, University of Kansas Press, Lawrence, Kansas.

This volume presents the proceedings of a Workshop on Frozen Tissue Collection Management, in which papers stressing the value of frozen tissue collections for studies in basic, applied and forensic sciences were presented, guidelines for collection management were discussed and a plan to promote and coordinate use, growth and funding of tissue collections was formulated. Contents include sections detailing the value of and need for, collections of frozen tissues, simple methods for collecting tissues under field conditions, knowledge on long-term stability of tissue components, U.S. federal regulations concerning tissue collection and transport and results of a worldwide survey of tissue collections holdings. This seminal volume is still of tremendous value to tissue collection managers, users and potential users and officials of all federal, state and private funding agencies. The directory of frozen tissue collections was updated in Dessauer et al. (1996).

Herholdt, E. M. (1990). *Natural History Collections: Their Management and Value*. Transvaal Museum Special Publication No. 1, Transvaal Museum, Pretoria.

This volume presents the proceedings of a symposium on the management and value of natural history collections held at the Transvaal Museum, Pretoria. Contributed papers deal with general aspects of the management of fluid collections, the conservation of natural history material, the computerization of collections, the importance of voucher specimens and the extensive uses of collections. Contents reflect the broad nature of collections management, the value of collections and the expertise involved in their curation. This volume serves both as a useful reference for museum curators and collections managers, by stimulating professionalism in collections management and as a primer on the value of natural history collections outside the scientific community.

Hoagland, K. E. and Rossman, A. Y. (1997). *Global Genetic Resources: Access, Ownership and Intellectual Property Rights*. *Beltsville Symposia in Agricultural Research 21*. Association of Systematics Collections, Washington, D.C.

This volume presents the proceedings of the Beltsville Symposium on Global Genetic Resources, which explored issues relating to ownership of and access to genetic resources and biological specimens. Laws are being implemented in countries throughout the world that severely restrict access to these research resources, thereby affecting the ability of scientists to pursue their goal of providing knowledge to benefit the world's people. Contributed papers examine the status of the various treaties, national laws and agreements in effect around the world, present case studies demonstrating how research using international resources benefit the global community, explore models of the equitable use of genetic resources and discuss potential solutions and mutually beneficial compromises. An Association of Systematics Collections position paper, based on the presentations, discussions and an open session immediately following the symposium, is also presented.

Zimmer, E. A., White, T. J., Cann, R. L. and Wilson, A. C. (1994). *Methods in Enzymology Vol. 224, Molecular Evolution: Producing the Biochemical Data*. Academic Press, San Diego.

This volume presents a compendium of excellent chapters, contributed by authorities, dealing with all aspects data acquisition and analysis for studies in molecular evolution. Guidelines for the collection and storage of samples are arranged into chapters on land plants, vertebrates, invertebrates, fungi and algae. Each chapter presents methods for the collection and temporary storage of freshly collected tissues in the field, as well as suggestions for alternative sources of tissues. Procedures for the transportation, long-term storage and documentation of samples, including the collection and deposition of voucher specimens, are provided. Each chapter also offers general information on the regulations permitting collection and transportation of samples. Subsequent chapters present protocols for isolation of proteins and DNA from fresh and ancient samples, including advice on the selection of tissues.