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-pre-

served 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

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.

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].

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

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 microcentrifuge 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].

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.

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 (Data-Works 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 for-

mats), 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

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.

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.

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.

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.

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

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].

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

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.

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