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SHORT ■ DIKOW ■ MOREAU

COLLECTIONS IN THE AGE OF BIG DATA

ENTOMOLOGICAL COLLECTIONS IN THE AGE OF BIG DATA

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■ **Abstract** With a million described species and more than half a billion preserved specimens, the large scale of insect collections is unequalled by those of any other group. Advances in genomics, collection digitization, and imaging have begun to more fully harness the power that such large data stores can provide. These new approaches and technologies have transformed how entomological collections are managed and utilized. While genomic

research has fundamentally changed how many specimens are collected and curated, advances in technology have shown promise for extracting sequence data from the vast holdings already in museums. Efforts to mainstream specimen digitization have taken root and have accelerated traditional taxonomic studies as well as distribution modeling and global change research. Emerging imaging technologies such as [microcomputed tomography-CT](#) and [Confocal Laser Scanning Microscopy](#) are changing how morphology can be investigated. This review provides an overview ~~on of~~ how the realization of [“big_data”](#) **AU: Quotation marks used for purposes other than direct quotation have been removed throughout, per house style.** has transformed our field and what may lie in store.

Keywords bioinformatics, biological specimens, digitization, genomics, natural history collections

INTRODUCTION

Natural history collections are in a state of rapid transition, perhaps more so than at any other time in their history (13, 75, 148). Once considered largely the domain of taxonomists and morphologists, biological collections have emerged as the scientific stage for a host of disciplines that rely on the breadth and depth of biodiversity captured in museums (133). [Biological collections are foundational across many research fields.](#) From documenting and describing populations, species, and their tremendous diversity; improving public health, agricultural practices, and food security; monitoring environmental contamination; and studying the effects of biological invasions and global climate change, ~~biological collections are foundational across many research fields~~ (29, 133). These transitions are driven largely along two fronts: first, the liberation of vast troves of specimen data that were previously available only to those who physically handled specimens; and second, innovations and technological advances in genomic sequencing and morphological imaging. Altogether, these developments have unleashed a torrent of data, the magnitude of which was almost unimaginable even a decade ago.

BIOLOGICAL COLLECTIONS ~~&~~ AND GENOMICS

Most material housed in entomological collections was collected prior to the routine generation and inclusion of molecular data in taxonomic and evolutionary studies—often

before DNA itself was ~~even~~ discovered. Consequently, most insect specimens were not collected with molecular and DNA-based studies in mind, and while many ~~contemporarily-collected~~ museum specimens are caught and preserved today using methods to minimize genetic degradation, this is by no means standard practice for a variety of practical, economic, and/or historical reasons. Since the majority of biodiversity represented in museum holdings is likely to be difficult to re-collect due to destruction of habitats, species and population loss, prohibitive costs ~~to~~ of revisiting field sites, and shifting collecting and export policies, the scientific community has increasingly turned to these existing museum collections as sources of genetic material.

Specimen Preservation and Storage

Entomological collections typically consist of a dry (pinned, pointed, or enveloped) collection, a wet or alcohol collection, and a slide-mounted collection. Historically, these types of ~~types~~ collections encompassed the vast majority of diversity contained in entomology collections and permitted a range of research techniques, including morphological examinations and dissections, even with very old samples. With a diversity of options available for preserving specimens, museum professionals are faced with often competing dilemmas on how to ensure collections have the highest scientific value now and into the future, while balancing time investment ~~time~~ and cost-effectiveness to care for the large collections that most museums house (78, 80).

The relative success of extracting DNA from museum specimens preserved in these traditional ways, and whether this genetic material is suitable for the study at hand, depends on many factors, many of which remain ~~poorly understood~~ ~~unknown~~ (65). Some molecular and genomic methods can take advantage of traditionally preserved specimens (i.e., Sanger gene-based sequencing, reduced-representation genome and locus sequencing, reduced-representation locus and genome sequencing ****AU: Is the difference in order significant here?*****) and assembly (Table 4), but new collections or alternatively preserved specimens are required for other genomic techniques and technologies (gene expression, ~~annotating~~ ~~genomes~~ annotation, whole-genome sequencing) (Table 1).

<COMP: PLEASE INSERT TABLE 1 HERE>

Recognizing the limitations of traditional preservation methods, almost three decades ago, many collection curators and other scientists who vouchered their specimens in museum collections began advocating for storage techniques that more readily facilitated molecular studies (17, 80, 149). Although it may therefore seem (Table 1) that all future museum collections should be preserved in high-grade ethanol, ultracold freezers, and/or cryogenic facilities (Table 1), these storage methods make the specimens more difficult to access, and are more costly to implement and maintain than traditional methods, and in most cases are likely cost-prohibitive for many small to medium-sized collections (22). Several networks have recently been created for preserving biodiversity for genomic methods, including the Global Genome Initiative (<http://ggi.si.edu>) and the Global Genome Biodiversity Network (http://www.ggbn.org/ggbn_portal/), although none specifically targets insects. Additionally, for groups such as Lepidoptera, new collection and storage protocols have been developed to optimize the better maximize use of specimens for both DNA and morphological work (19). This balance of benefits and costs requires museum professionals to prioritize collections for preservation media, which is the model most collections currently follow.

Many institutions have adopted policies for destructive and nondestructive sampling of accessioned specimens. Although some permit destructive methods for sampling DNA, such methods preclude preservation of the lack of a voucher and the single-use nature of such potentially irreplaceable specimens is safe not desirable [AU: Reviewer Comment: As meant?]. Fortunately, several nondestructive or minimally destructive methods for extracting genetic material have been developed for insect specimens (18, 45, 127, 138, 139). These methods do not generally require damage or trauma to the exterior of the specimen, though although soft tissues may be lost in the process.

We would argue here that rare specimens and nonmodel and rarely studied groups of organisms should be preserved in a manner that increases the likelihood they can be studied into the future. Regardless of storage preservative or method, tissues or whole specimens must be carefully entered into databased-databases and labeled to ensure the highest standard for research utility.

Museum Specimens and Sanger-Based Sequencing

Gene-based sequencing using Sanger sequencing technology has greatly advanced understanding of the ~~T~~ree of ~~L~~ife during the last several decades. Several studies have considered the utility of traditional museum storage techniques for DNA-based studies of insects (28, 38, 39, 72, 94, 108, 109, 110, 141) and insect-associated microbiota (47, 94) using Sanger sequencing, which has less stringent specimen preservation requirements than many modern genomic methods (Table 1).

Although the choice of gene(s) that can be targeted depends on the age of the group of study, in many groups of insects and other arthropods, the number of available primer sets is restricted, leaving researchers with a limited choice of markers. In fact, for most DNA barcoding studies in insects, only a single mitochondrial gene fragment, cytochrome oxidase I (~~COI~~) **Acronyms used fewer than two times in the main text have been removed throughout, per house style.** is included (48, 125), ~~an approach that which~~ has come under criticism in the scientific literature (59, 83, 95, 126). Although there are concerns about the broad utility of DNA barcoding, this method has been successfully used on DNA obtained from ~~museum~~ insect and other arthropod museum specimens (51, 89, 127). The small number of developed primer sets can be viewed as a limitation, but the limited number has also permitted the combination of independently developed data sets **Edited to two words per Webster's without tracking below** to produce a broader understanding of the phylogenetic relationships of groups of arthropod taxa; examples include beetles (58), centipedes (46), ants (12, 93), and butterflies (145). In addition, for researchers who are interested in adding one or a few new samples to an existing phylogeny, generating the Sanger gene-based data to include these new taxa in legacy data sets ~~will is still be~~ the best option based on time and cost.

Another approach that relies on Sanger sequencing or 454 pyro-sequencing but produces significantly more data than the gene-based approach, is the creation of an expressed sequence ~~tag~~ (EST) databases **Reviewer Comment: As meant?** (ESTs). ESTs can be used to help annotate genomes, but ~~they~~ have also been used successfully to reconstruct the phylogenetic relationships within several

arthropod groups [e.g., beetles (57); Polyneoptera and Paraneoptera (73); arthropods (84); Hymenoptera (121)]**[[**AU: This text-plus-reference citation style has been reformatted per house style as shown here without tracking below**]]**. Although ESTs have been phylogenetically informative, the much higher cost and time investment incurred to generate these data when compared with newer technologies suggests this approach may be short lived ~~may not see continued interest~~ for phylogenomics.

Museum Specimens and High-Throughput ~~(HT)~~ Sequencing

Reduced-representation genome sequencing (RRGS) protocols using next-generation and high-throughput sequencing are providing large-scale multigene/multilocus data sets. These methods, including restriction-site-associated DNA sequencing (RAD-seq), genotyping-by-sequencing (GBS), targeted sequence capture/hybrid enrichment, transcriptomes for gene capture, and highly conserved or ultraconserved elements (UCEs), are proving valuable for inferring insect phylogenies [e.g., beetles (65); butterflies and moths (7, ~~66~~ 67); wingless insects (27); ants, bees, and wasps (36, 62); lower neopterans (74); insects 909 **[[**AU: Please resolve reference**]]**; holometabolous insects (104); polyneopterans (123)]. As these methods rely on high-throughput sequencing, which targets shorter fragments than traditional Sanger sequencing, some researchers have been able to employ them to sequence museum specimens that have been traditionally preserved ~~in traditional ways~~, resulting in DNA that is frequently highly fragmented (98, 138).

A few studies have explicitly compared RRGS data with the traditional Sanger gene-based ~~Sanger~~ sequencing [e.g., beetles (24), ants (11)], demonstrating the utility and power of these approaches. One concern ~~for~~ with some of these methods is the level of evolutionary divergence between the included taxa. For example, through a simulation study based on real genome data from fruit flies and other taxa, Rubin et al. (114) demonstrated that RAD-~~S~~seq and GBS-genotyping-by-sequencing methods are informative for phylogenetic inference for clades younger than 50 million years (Mya). This drop-off in utility at deeper timescales is due to mutations in the restriction sites that resulting in loci not being sequenced for some taxa and uncertain homology of short fragments with many nucleotide substitutions. Targeted capture and UCEs may suffer less from this specific problem depending on the capture

design and probe set developed, which can be targeted to include loci with differing evolutionary rates. ~~Further~~ Furthermore, UCE probe sets can be developed for very specific questions [e.g., from genus- or even species- to order-level phylogenies (61)] and can be resequenced to generate compatible data sets for other lineages or to add new taxa to an existing ~~one~~ lineage, similar to Sanger-based data sets.

The expansion of ~~As~~ molecular systematics ~~into~~ ~~has entered~~ the genomic era (140) ~~this~~ may broaden the usefulness of traditionally preserved material, which represents the bulk of existing museum collections. Although RRGs methods are proving highly informative in phylogenetic analyses and may permit inclusion of traditionally preserved museum specimens, the bottleneck for many RRGs data sets is bioinformatic in nature, requiring the ability to manipulate and analyze the ever-growing data sets efficiently. Accordingly, ~~s~~ students of entomology, ecology, and evolution need to be well-trained in bioinformatics and big-data analysis techniques.

Genome Sequencing, Comparative Genomics, and Gene Expression

The number of insect genomes and molecular data sets increases each year (Figure 1), providing the opportunity to ask novel questions about the evolution, physiology, gene function, and development of a diversity of nonmodel organisms. Although most genome approaches require specimens that have been preserved with nontraditional methods (Table 1), traditionally preserved museum insect specimens have been used for mitochondrial genome sequencing. Due to their smaller size and higher copy number per cell, mitochondrial genomes are ideal for capture from DNA obtained from museum specimens. Timmermans et al. (137) were able to sequence the mitochondrial genomes of 35 butterflies using museum specimens, and Staats et al. (128) sequenced the mitochondrial genome of two fly species (*Aedes albopictus* and *Ceratitis capitata*) and one beetle (*Anoplophora glabripennis*) species from museum specimens. Although no entire nuclear genome has yet been sequenced from a traditionally preserved museum insect specimen due to the degraded nature of their DNA, this will likely change as sequencing technologies improve. As recovering mitochondrial genes and genomes ~~is~~ are more likely to occur when ~~from~~ museum specimens are used, Cameron (14) advocates for mitochondrial genome sequencing to serve

as a bridge between legacy and contemporary data sets- given thatas these genomes are by- products of almost all high-throughput sequencing genome projects.

<COMP: PLEASE INSERT FIGURE 1 HERE>

Figure 1 Number of insect species with genomes ~~on~~in the Genbank database from 2002 through 2016. ["**AU: It is the author's responsibility to obtain permissions for figures being adapted or reprinted from previous publications. Please obtain permissions as necessary and add permissions verbiage to figure captions where applicable.**"]

In fact, of the 304 insect genomes available ~~on~~in the National Center for Biotechnology Information (NCBI) GenBank (<http://www.ncbi.nlm.nih.gov/genbank>; accessed ~~12~~February 12, 2017), 46 species (20%) have at least one author with a museum institutional affiliation. Museum genomics is still in its infancy, but carefully designed practices for the preservation of genetic material ~~are poisoning~~have poisoned museum collections to take a place at the forefront of biodiversity genomics (30).

Host-Associated Microbiomes and Metagenomics

Microbes can have profound effects on their hosts, from negative interactions in the form of pathogenesis and parasitism to highly beneficial associations in the form of nutrient provisioning and immune defense (32, 64, 82, 91, 97, 131). Identifying which arthropods harbor microbes and characterizing the diversity of ~~the~~ microbes that are specialized partners with their hosts is still at an early stage (21, 63, ~~6766~~, 81, 105, 115, 118, 147). With large numbers of identified specimens, museum collections are likely to have~~make~~ a significant impact in elucidating~~understanding~~ host-microbe partnerships, because most community bacterial amplicon sequencing ["**AU: "amplicon sequencing of bacterial communities"?"**] targets short stretches of microbial DNA ["**AU: Reviewer Comment: Cite reference?"**] and museum collections house millions of specimens that could be explored for their host-associated microbes.

In addition to determining the diversity of microbes present in or on a host, understanding their function is key to comprehending their roles in host health and productivity. Sequencing the genomes of host-associated microbes can be highly informative, but has historically ~~has~~ required the ability to isolate or culture each species ~~/or~~ strain (16, 26, 54, 92, 134). Metagenomics ~~is~~ can serve as a tool to

sequence the genomes of microbial partners found in a host or ~~in a~~ specific location in or on a host and has been informative for elucidating the functions of microbes across diverse insects and other arthropods (3, 34, 146). ~~Since~~ ~~U~~University and museum collections house the broadest sampling of insect diversity, ~~these collections~~ ~~and~~ are ~~thus~~ certain to make significant contributions to ~~ward~~ understanding host-associated microbial interactions, although the ~~ir~~ full utility ~~of such collections~~ in this field has not been realized to date.

Metagenomics: a technique in which DNA sequencing is used to study genetic material obtained from community sampling ****AU: Definition has been adjusted to be consistent with how the term is used in the text above. OK as done? ****

COLLECTIONS DIGITIZATION ~~&~~ AND INFORMATICS

The size and complexity of entomological collections, and consequently the magnitude of the task in digitizing them, ~~are is~~ unrivaled in natural history collections. For example, the federated database VertNet contains ~~approximately~~ ~18 million digitized records for vertebrate specimens from more than 300 collections. The National Museum of Natural History, Smithsonian Institution, alone is estimated to contain >34 million insect specimens, of which only 421,698 records are available online ~~at through the~~ **GBIF** (<http://www.gbif.org>; see ~~also supplementary~~ ~~Supplemental~~ ~~Materials~~). The challenge is matched by the opportunities that the liberation of these data can unlock. The diversity of research questions that can be addressed is enormous, and ~~entomologists~~ ~~we~~ have only begun to unleash the power of these data (e.g., The Atlas of Living Australia, <http://www.ala.org.au>).

GBIF: Global Biodiversity Information Facility, a public repository for species occurrence data

Efforts to digitize collection objects ~~---here~~ defined ~~here~~ to include data capture and/or imaging of either individual specimens or “management units” such as drawers, vials, and slides (and not simply lists of museum holdings) ~~---now~~ play a major role in the curation of many natural history museums. Digitization of entomological collections is now firmly in its third decade ****AU: Reviewer Comment: Might there be a historical record to cite here, from the 1990s? Is Norman Johnson’s 2007 Annual Review on biodiversity informatics useful**

[here?*](#). However, it is only in the last five to ten years that the practice [has](#) expanded from early-adopting collections to become fairly widespread in the community. At the same time, what it means to “digitize” collections has evolved and diversified, often depending on the objectives and [available](#) resources [available](#) underpinning the effort.

Specimen-Level Data Capture

Traditional specimen-level data capture (SLDC) has been and remains the gold standard for the digitization of insect specimens. We define SLDC to include at a minimum [the: \(1a\)](#) ~~the~~ physical labeling of individual specimens with a human- and/or machine-readable unique specimen identifier; [\(2b\)](#) ~~the~~ transcription [\[**AU: Reviewer Comment: I am not sure the general reader will know that, by “transcription,” you mean the recording of label data into a database. How about “the transcription of the specimen labels, including taxonomic identification, into a database, . . .”?*](#) of the specimen labels, [including](#) ~~and its~~ taxonomic ~~identification~~ ~~identity~~; and [\(3c\)](#) ~~the~~ parsing of these digital data into appropriate data standards such as **Darwin Core**. Georeferencing, while not a necessity of basic SLDC, significantly increases the value of specimen records. When shared via publicly accessible web portals such as [the](#) GBIF (<http://www.gbif.org>), specimens and their associated data ~~that were~~ digitized using SLDC protocols become discoverable to the global scientific community and other stakeholders. These records [can](#) not only ~~can~~ assist traditional users of museum specimens [in](#) ~~discover~~ [ing](#) holdings of interest; but [also](#) permit research on environmental change, public health, invasive species, conservation, and a host of other topics [\(4\)](#). That being said, data cleanliness remains a significant issue inhibiting the application of biodiversity data that needs to be addressed [\(77\)](#).

Darwin Core: a set of controlled vocabularies for biodiversity data and collections

Imaging-based approaches for semiautomated digitization of individual specimens or slides are also being developed. One example, [the](#) GIGAMacro Magnify² system (<http://www.gigamacro.com/gigapixel-macroimaging-system/>), includes robotic automation, image capture, postprocessing, online viewing, sharing, and annotation. This system enables the user to set-up 36 pinned or 84 slide-mounted specimens to be imaged in a single session. The position of each specimen

or slide is registered by the operator in the included software and the system will proceed unattended to take images of each specimen in the *X*-, *Y*-, and *Z*-axes. These images are stitched together and stacked and the final image can have gigapixel resolution. Pinned specimens can then be rotated for automated imaging of other features in different views.

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Digitizing in Bulk

In recent years, whole-drawer imaging (WDI) of entomological collections has become a growing trend. Rather than imaging or data-capture of individual specimens, WDI involves creating an image of the entire specimen drawer (6, 49). While not a replacement for specimen-level digitization, WDI efforts have been driven by the increasing realization that there are no shortcuts to SLDC, and the relatively quick, cheap, and unique benefits WDI provides (53). While in principle WDI can be done with a single photograph, such images would provide scant resolution ~~on~~ for individual specimens. Consequently, composite images are created by stitching together many (often hundreds) of image tiles, each representing a small fraction of the drawer. These scalable composite images often allow even the smallest insects to be viewed with a relatively high level of detail, and ~~read~~ any label information that may be visible from above to be read.

Early approaches to whole-drawer digitization adapted existing technologies from panoramic photography such as GigaPan to image whole drawers (8). Here, a camera is suspended above the object and swivels to take several images in the *X*- and *Y*-axes that are stitched together for a final, ultrahigh-resolution image of the entire object. The parallax effect, unfortunately, is still apparent and images will be distorted and less accurate towards the edges. Other recent efforts such as SatScan Collections (10, 79), DScan (120), and Invertnet (28) have worked to build more customized hardware for automated digitization of an entire drawer containing specimens or a tray of slides. In SatScan and DScan, the camera is moved horizontally and takes several images in the *X*- and *Y*-axes of the entire object, ~~which~~ that are stitched together to provide a final, ultrahigh-resolution image of the entire drawer. The parallax effect of regular photography is eliminated in both systems. The SatScan integrated software allows the user to add metadata. In the robotic system developed by InvertNet (28), the camera is situated in the center and takes several

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hundred images in the *X*- and *Y*-axes of a drawer that are stitched together and resulting in a final, ultrahigh-resolution image of the entire object. The novelty of the InvertNet system is that the camera is also tilted to take overlapping images from various oblique angles. The individual pinned specimens can now then be “examined” in dorsal view and in-oblique views so that the label data become more easily readable. Through online transcription by amateurs or scientists, data about individual specimens in the image can be transcribed and added to a specimen-level database (52).

Various software packages have been developed to accommodate the needs of digitizing insect drawers. Hudson et al. (56) provide a modular, cross-platform suite of open-source software tools, called *Inselect* **AU: Boldface and italic used for emphasis have been removed throughout, per house style.** *Inselect*, to automatically identify and isolate specimens from an imaged drawer. *Inselect* allows the user to add metadata to the single-specimen images. While obtaining specimen-level data is an important goal, the label data and unique specimen identifier label might not be visible resulting from a SatScan Collections or DScan drawer image. InvertNet developed their hardware and software suite to take the explicit specimen-level data capture into consideration by imaging specimens and labels in several angles. GIGAMacro, GigaPan, and InvertNet provide a custom image-viewing solution on their respective platforms, while whole-drawer images from SatScan Collections and DScan can be viewed in a variety of available viewers. The Australian National Insect Collection provides access to the whole-drawer images in Morphbank (<http://www.morphbank.net>). There are other custom imaging solutions available in natural history museums, such as pinned insects photographed on a rotating platform, which that can be viewed from all sides (and are accessible at ZooSphere, (<http://www.zoosphere.net>)).

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Field to Database: Digitization Beyond Specimens

Data associated with museum specimens are often not limited to what is printed on the labels. Original field notes, images of habitat, or recordings of sound or behavior are just a few of the rich resources that may be included in research collections. The existence of these materials is often unknown to other researchers and they are typically less

discoverable than the specimens themselves, if at all. The inclusion of these materials in digitization efforts was virtually unheard of a decade ago, but is now increasing in prevalence. Some efforts, such as the Field Books Project (99), have focused on the digitization and annotation of original field notes, sketches, maps, and images. Others, such as the Collections Resources in Aquatic Coleoptera project (CREAC, <http://creac.kubiodiversityinstitute.org/collections/>), emphasize not just the digitization of these materials, but also their association with the specimens themselves (if they exist) in a single integrated platform. The unification of specimens with their original field notes and images facilitates the detection of labeling errors, more accurate georeferencing, and insights into the taxon's biology. [The data standard Audubon Core \(96\)](#), ~~a set of controlled vocabularies for biodiversity data associated with multimedia resources,~~ **[**AU: Definition has been removed from text in favor of the one that will appear in the margin on the same page \(and be hyperlinked in the online version\) to avoid repetition. This is the only occurrence of the term. OK as done?**\) is available to facilitate the sharing of many kinds of these data.](#)**

Audubon Core: controlled vocabularies for multimedia such as images and videos associated with biodiversity data

Uses of Digitized Collections Data

Despite considerable SLDC efforts in the last 20 years, we estimate that ~~fewer~~ less than 2% of insect specimens in museum collections have been digitized (see [supplementary Supplemental Materials](#)), highlighting both the magnitude of the task ~~ahead we face, as well as~~ **[and](#)** the enormous amount of data yet to be made publicly available. Despite the long road ahead toward complete digitization, the increase in quantity, quality, and availability of data from SLDC in the last decade has already substantially expanded the reach of collections data. Ecological niche modeling in particular has emerged as a powerful tool capable of utilizing the large troves of distributional data ~~that~~ specimens provide to detect past changes and predict future trends in the ranges of insects (e.g., 19). Bees have been a premier exemplar of how concerted efforts to digitize specimen data for a particular taxon can yield powerful insights into species distributions (68), the effects of environmental change (15, 70), and evolution (122). Digitized field notes have even facilitated the

discovery of insects once thought to be extinct (154^[**AU: Reviewer Comment: Please confirm this reference (Yoder et al.).**]).

Large-scale imaging of collections, most notably with WDI, have afforded taxonomists and the general public the ability to browse museum collections from their offices or homes. It allows taxonomists to discover holdings of interest that previously would have never have been studied. For some taxa, it can facilitate preliminary sorting or identifications without the need to ~~send-transport~~ the material, reducing the risk to the specimens.

MORPHOLOGY: THE CUTICLE AND BEYOND

The morphological study of insects, which relies heavily on specimens in entomological collections, has seen dramatic changes over its more than 260+-year history (9, 44). Probably the biggest boost to morphology outside of improved microscopes was the advent of digital, three-dimensional (3D) reconstructions of morphological features (25, 44). Such 3D data have been used for deciphering the phylogenetic relationships of insect taxa (e.g., 9, 41, 42, 43, 55, 104), obtaining additional data from amber and compression fossils (e.g., 1, 50, 103, 106, 119, 135), examining internal anatomy (e.g., 35, 40, 69, 87, 88, 107, 127, 155), investigating internal musculature used for power-amplified movements (e.g., 152), and describing new species in taxonomic revisions (e.g., 37, 85, 86, 130). However, Deans et al. (25^[**AU: Please include page number of quote**]) highlight that “Internal anatomy remains a largely untapped resource for evidence of taxonomic association and evolutionary history” and call for expanded use of modern techniques that provide access to internal features.

The raw data for 3D reconstruction and visualization originate from a diversity of methods such as X-ray computed tomography ~~on~~(CT), micro-CT (μ CT), and synchrotron devices^{[**AU: Reviewer Comment: CT using synchrotron devices?]**}, laser ablation tomography, confocal laser scanning microscopy (CLSM), ~~or~~and traditional semi-thin cross-sectioning of embedded objects followed by digitization and stacking of the individual slices (for an overview, see 44). While ~~micro-~~ μ CT, synchrotron devices, and CLSM are nondestructive methods, laser ablation tomography and thin-sectioning are destructive methods. Specimens with

thorough 3D reconstructions can be virtually dissected on a computer screen to reveal minute and internal structures. ~~This, which~~ is especially useful for the study of very small insects that cannot be dissected with traditional methods (e.g., 152). All of these new imaging advances capitalize on the vast existing holdings of collections, providing the ability to examine larger numbers of characters at levels of detail that were previously intractable. While ~~for some applications~~ specimens should be preserved in special ways ~~for some applications~~ (44), pinned and ethanol-preserved specimens that make up the vast majority of existing insect collections can be used to gather novel morphological data with the above techniques.

~~3D-Three-dimensional~~ reconstructions can play an important role in teaching and outreach by bringing the study of insects and detailed morphological analysis to students and the public at large through classroom and museum exhibits with interactive displays.

Microcomputed Tomography-CT (μ CT)

In 2002, Hörnschemeyer et al. (5455) published one of the first morphological phylogenetic studies using μ CT data to elucidate the position of the cupedid beetle *Priacma serrata* within Archostemata (Coleoptera). Since then, the available resolution has been enhanced to 0.1 μ m [nano-CT (9)]. With ~~micro- μ CT~~ and, to a lesser degree, nano-CT scanners becoming more easily accessible for research purposes, ~~in the near future~~ 3D reconstructions will become commonplace in taxonomic, systematic, and evolutionary studies in entomology ~~in the near future~~. Because of its nondestructive nature, ~~micro- μ CT~~ scanning can advance the study of rarely collected or unique type specimens (9). It also works well for fossils and in particular for amber fossils because, while fossils can be scanned with **synchrotron** sources (135), the high energy used in synchrotrons can damage fossilized resin.

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Confocal Laser Scanning Microscopy (CLSM)

For the study of external morphology, CLSM takes advantage of the autofluorescence of the cuticle (9, 71). Small insects or parts thereof can be studied easily, but internal structures of larger insects can ~~only~~ be examined ~~only~~ after clearing the cuticle (9). In contrast to ~~S~~scanning ~~E~~lectron ~~M~~icroscopy, CLSM provides the opportunity ~~of for~~ 3D

reconstruction and visualization of small structures. While there is potential for signal loss artifacts, Klaus et al. (71) provide details on how to overcome them.

Managing and Sharing Data

Data standards are increasingly important for making the sharing and reuse of gathered morphological data a reality (143, 144). Media files in particular require associated metadata so that they can be stored in databases and made accessible to the community. Ontologies, defined and formalized vocabularies of terms and relationships (142, 154), ~~similarly~~ ~~likewise~~ enhance ~~the~~ ~~our~~ ability to apply previously gathered morphological data to questions in both entomological science and research fields outside of entomology. Balhoff et al. (53) ~~**AU: Balhoff is not Reference 53. Should this cite the presently uncited Reference 5 queried in Literature Cited?**) provide an example of combining cybertaxonomic tools with annotating character and character-state combinations with the Hymenoptera Anatomy Ontology (154).~~

New imaging methods have necessitated the development of new and novel publishing tools. Authors are now able ~~to~~ not only ~~to~~ include static representations of 3D reconstructions such as images in manuscripts; but also ~~to~~ embed videos and other media that can be interactively viewed by the reader in the article PDF (116, 117). In the published PDF, Faulwetter et al. (37) include videos derived from μ CT data of the volume renderings of a polychaete worm for the virtual dissection and surface models for viewing a structure from all angles. The reader can interact with the video and study the virtual type material, called a “cybertype” by Faulwetter et al. (37), in the desired position. Ernst et al. (35), Mikó et al. (88), and Popovici et al. (107) provide access to the interactive volume renderings and surface models derived from CLSM through a data depository, and the individual videos are identified by Digital Object Identifiers (DOIs) in article PDFs for easy retrieval.

Big data gathered through μ CT and CLSM highlight the critical need for mechanisms for utilizing and sharing scientific data. Data depositories from which information can be retrieved and reanalyzed have a long history in the biological sciences; NCBI’s GenBank is probably the most common depository used by entomologists. Guidance for locating a depository can be found in Whyte (150).

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Cranston et al. (23) provide ten simple rules on best practices for data sharing in taxonomic and phylogenetic research. Just like other raw data, 2D (pixel) and 3D (voxel) imaging data should be made accessible and uploaded to dedicated, open-access depositories. Rowe and Frank (113) emphasize that 3D image data drastically lag behind in archiving and that at the time only two depositories, DigiMorph (<http://www.digimorph.org>) and Digital Fish Library (DFL), were suitable for archiving 3D voxels as raw data. This situation has not changed, unfortunately. Dedicated 2D image depositories such as Morphbank (<http://www.morphbank.net>) enable the user to add metadata, and several others such as MorphoBank (100) also enable the use of ontologies. General depositories such as Dryad (<http://www.datadryad.org>), FigShare, or Zenodo, all of which provide Digital Object Identifiers (DOIs) for each data set, can be used for archiving raw 3D data, volume renderings, and surface models so that they can be cited in a publication.

With the tools available to gather and store data, it is now incumbent upon the community to adopt the sharing of raw and edited data to stimulate vigorous scientific debate through testing previous hypotheses and erecting new hypotheses [**AU: Reviewer Comment: Can one test data? data (41)]. GenBank has been so successful for storing and providing access to molecular data for decades because it was a standard that was adopted by the community and the morphology community needs to follow [this example](#).

FUTURE ISSUES

1. In addition to traditional preservation methods, more museum collections are archiving entomological specimens with future genetic and genomic uses in mind. However, inconsistent data sharing currently inhibits the discovery and ~~optimal~~ maximal utilization of these resources. Efforts to digitize and network these genomic resources, such as the Global Genome Biodiversity Network, would greatly benefit the community.
2. Recent and emerging genomic approaches are increasingly able to utilize historical museum specimens whose DNA may have been too fragmented for traditional Sanger-sequencing methods in the past. While there have been many successes, there remains a

need ~~for deeper understanding and more accurate quantification of~~ ~~to better understand and quantify~~ the factors that affect the suitability of genomic DNA ~~of~~ ~~from~~ historical specimens.

3. With only 2% of entomological specimens estimated to have been individually digitized, new workflows for SLDC must be developed and/or additional resources must be allocated to increase the rate of data dissemination from entomological collections.
4. ~~The prevalence of the d~~Digitization and integration of specimen-associated collecting data such as field notes, habitat images, and vocal recordings ~~is~~ ~~are~~ likely to ~~rapidly increase as~~ ~~expand rapidly as these practices are~~ ~~it is~~ adopted by more entomological collections. New protocols are needed to ensure these data are fully integrated with SLDC practices.
5. Current and emerging methods to digitally capture insect morphology both internally and externally provide the opportunity to establish new data sources for taxonomy and phylogeny. Making these data accessible through open depositories and using character ontologies can provide the basis for comparative evolutionary studies across diverse taxa ~~currently that are~~ not ~~currently~~ possible ~~today~~.
6. ~~As~~ ~~Because~~ museum collections may in some cases ~~be~~ ~~contain~~ the only record of a species or ~~the~~ specific geographic location of a species/population and new technologies are permitting broader uses of these collections, it is imperative that institutions and governments continue to invest in and support these invaluable archives of biodiversity.

DISCLOSURE STATEMENT

*****AU: Please insert your Disclosure of Potential Bias statement, covering all authors, here. If you have nothing to disclose, please confirm that the statement below may be published in your review. Fill out and return the forms sent with your galleys, as manuscripts CANNOT be sent for page proof layout until these forms are received.*****

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Table 1 Museum storage methods and utility in DNA and genomic research

	Gene-based DNA sequencing, DNA barcoding, & and ESTs	Reduced-representation genome sequencing (e.g., RAD-seq, UCEs)	Genome sequencing & and assembly	Genome and gene annotation	Gene expression and transcriptomes	Host-associated microbes	SourcesReference(s)
Dry/pinned storage	Some utility	Some utility	Some utility	No	No	Some utility	45, 65, 128, 132, 138
Medium-grade (70%) Et ethanol	Some utility	Some utility	Some utility	No	No	Some utility	129
Propylene glycol	Some utility	Some utility	Some utility	No	No	Some utility	94, 129
High-grade ethanol (95--100%)	Yes	Yes	Yes	Some utility	No	Yes	94, 109, 129
-20°C freezer	Yes	Yes	Yes	Some utility	No	Yes	2
-80°C ultracold freezer	Yes	Yes	Yes	Yes	Yes	Yes	2, 109
RNA preservative	Yes	Yes	Yes	Yes	Yes	Yes	94, 109
Cryogenics/liquid nitrogen	Yes	Yes	Yes	Yes	Yes	Yes	109

[Note that](#) Some methods for which utility has been found may [only](#) be [only](#) for short- or medium-term durations, rather than permanent archival storage. [Abbreviations: ESTs, expressed sequence tags; RAD-seq, restriction site-associated DNA sequencing; UCEs, ultraconserved elements.](#)

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