Chapter 25
Molecular Tools for Assessing Saproxylic Insect Diversity

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Abstract Little is known about the amount and spatial distribution of diversity within and among deadwood-dependent insect species and saproxylic communities as a whole. Molecular approaches offer a solution to these knowledge gaps, even in cases where species and genera are not yet formally described. Indeed, molecular data are broadly connectable among otherwise unrelated studies and directly complement the invaluable work of expert taxonomists. Here we provide an overview of the applications of molecular tools for assessing saproxylic insect diversity. To do this, we use an organizational framework based on the hierarchy of biological units, beginning with diversity at the intraspecific level, followed by species-level diversity within genera, and then close with community-level diversity. Within each of these sections, we consider the types of genetic data that have typically been used and provide an overview of research questions and findings from the primary literature.

25.1 Introduction

Deadwood-dependent (saproxylic) insects are an ecological community that exhibits considerable diversity across different levels of biological organization, from populations to species and beyond. This group also encompasses an array of life history traits relating to metamorphosis, reproduction, dispersal, longevity, and feeding. Furthermore, saproxylic insects are ecosystem service providers that contribute to the decomposition of fallen trees and thus play roles in maintaining healthy, productive forests (Ulyshen 2013, 2014, 2016; Ulyshen and Wagner

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2013). While these beneficial services extend to commercial forests and unmanaged forests, saproxylic insects are an overlooked component of biodiversity (Speight 1989; Siitonen 2001; Yee et al. 2001; Grove 2002a; Garrick et al. 2006) such that their needs are usually not explicitly integrated into natural resource management or conservation plans.

Although some saproxylic insects are capable of long-distance colonization (Ranius et al. 2011), as a group they typically have poor dispersal abilities (e.g., due to winglessness and/or rapid desiccation as a consequence of adaptations to life in a rotting log; Schmuki et al. 2006a, b; Garrick et al. 2012). Accordingly, this group can be particularly sensitive to human activities that cause forest fragmentation or prevent the natural occurrence of fallen trees (Schiegg 2000; Bouget et al. 2014; Seibold et al. 2015). Indeed, a disproportionately large number of threatened or endangered arthropods depend on deadwood (Jonsson and Kruys 2001). This has led some to suggest that saproxylic insects may be an early warning indicator for assessing forest health (Langor et al. 2006). However, a critical first step is to document the amount and spatial distributions of diversity in this poorly known group.

Two major challenges to documenting the diversity of saproxylic insects exist. First, many species and genera are unnamed, and there are too few trained experts to accomplish the task of describing them in the near future (i.e., the taxonomic impediment; New 1999). The sheer diversity of tropical saproxylic insects makes them taxonomically challenging in their own right (Grove and Stork 2000). Second, due to morphological conservatism of some groups or convergent adaptations (e.g., dorsoventrally flattened bodies, reduction or loss of eyes and pigment), many named species may actually be a complex of several cryptic species. This has been demonstrated repeatedly by molecular analyses of deadwood invertebrates (e.g., Trewick 2000; Walker et al. 2009; Oliveira et al. 2011). Consequently, for saproxylic insects, traditional biodiversity metrics based on morphologically identifiable named species, such as richness and turnover (i.e., alpha and beta diversity, respectively), will likely be downwardly biased. Indeed, the magnitude of this downward bias, and the extent to which it varies across a landscape, represents a knowledge gap in itself. Furthermore, traditional biodiversity metrics do not contribute information that can be used to protect unnamed species.

Molecular tools provide a pragmatic solution to the taxonomic impediment, to the design of large-scale monitoring schemes, and to the existence of cryptic species. For instance, distinct lineages can be defined on the basis of DNA sequence similarity, and given that the underlying data can be connected across distantly related groups, additional information on evolutionary relationships can be obtained. In addition to providing opportunities for less biased measures of local biodiversity in saproxylic insects, DNA sequence data also enable preliminary classification of specimens to named species [e.g., via mitochondrial DNA (mtDNA) barcoding approaches; Hebert et al. 2003]. Furthermore, molecular approaches can reveal specimens with divergent sequences that should become the subjects of focused morphological examination by expert taxonomists (Hebert and Gregory 2005; Hajibabaei et al. 2007).

Below, we give an overview of past and potential future applications of molecular tools for assessing saproxylic insect diversity. To do this, we use an organizational framework based on the hierarchy of biological units, beginning with diversity at the intraspecific level, followed by species-level diversity within genera, and then closing with community-level diversity. Whereas the former two categories benefit from a solid body of existing literature that specifically focuses on saproxylic insects, the latter category does not. Accordingly our treatment of community-level diversity draws from related literature (e.g., studies on saproxylic fungi) in order to look to the future of insect studies, and we propose a general research pipeline that may facilitate progress and/or stimulate debate. Finally, we briefly consider emerging directions in the use of molecular data to address questions about diversity and function in saproxylic invertebrates.

25.2 Specimen Sampling and Preservation

For the purpose of DNA extraction, tissue samples from saproxylic insects have been obtained in several ways. A common collection method has been carefully breaking open logs (e.g., using a small axe) and then visually inspecting the woody material for target taxa (e.g., Schmuki et al. 2006b; Leschen et al. 2008; Garrick 2017; Ulyshen et al. 2017). Occasionally, this approach has been augmented by using a Berlese funnel or Winkler sack to further process crumpled debris (Marske et al. 2009, 2011). Some studies have employed nonlethal sampling (e.g., only taking a clipping from a middle leg; Oleksa et al. 2013; Drag and Cizek 2015; Drag et al. 2015), which may have minimal impacts on survivorship and reproduction (Suzuki et al. 2012; Oi et al. 2013). More often, however, lethal sampling of whole specimens has been conducted, presumably because this enables morphological and other data to be collected, as well as vouchering of reference material.

As an alternative to dismantling rotting logs by hand, for some saproxylic beetles, pheromone-baited flight intercept traps or window traps have been used to sample specimens for molecular work (e.g., Svensson et al. 2009; Oleksa et al. 2013, 2015; Zauli et al. 2016; Harvey et al. 2017; Ulyshen et al. 2017). Although this approach avoids destroying deadwood microhabitats and may improve sampling efficiency when working on rare species, depending on the research question at hand, there are some issues that warrant consideration. For example, samples will be sex-biased if the pheromone is attractive only to males (e.g., Oleksa et al. 2015). Furthermore, depending on the speed of response and the duration of exposure, sampled individuals may travel relatively long distances before arriving at the trap, which would compromise the accuracy of geographic coordinates associated with trapped specimens. Finally, aside from pheromone traps being applicable only to flight-capable saproxylic insects, the timing of their deployment must generally coincide with reproductive phenology, which may be poorly known or geographically variable.

When planning for subsequent DNA analyses, ethanol has most often been used to preserve sampled specimens. However, in the context of trap-based collections,

evaporation might decrease the preservation property of ethanol under field conditions. In these cases, ethylene or propylene glycol might be an alternative. To obtain optimally preserved insects, Gossner et al. (2016) suggested using ethylene glycol instead of Renner solution (ethanol and glycerine) or copper sulfate, since the former solution had preserved samples better in a variety of microclimatic situations (also see Dillon et al. 1996). Propylene glycol might be used as a less toxic alternative (Höfer et al. 2015). Also, Pokluda et al. (2014) recommended using a solution of laboratory chemicals (i.e., 2% sodium dodecyl sulfate and 100 mM disodium ethylenediaminetetraacetate) as a cheap, stable, and easily transportable alternative to ethanol. However, its attracting effect has not been tested, and so potential biases in sampling for community-level studies remain unknown.

Once specimens have been collected and preserved, DNA extractions may be performed using nondestructive methods so as to preserve morphological characters. Protocols that yield sufficient amounts of genomic DNA from a broad range of terrestrial arthropods, and for which the specimens remain suitable for imaging and as vouchers, have been developed (e.g., Rowley et al. 2007; Castalanelli et al. 2010). Alternatively, if specimens are relatively large, destructive sampling of one or a few legs taken from the same side of the body should also retain morphological information.

25.3 Intraspecific Diversity

25.3.1 Genetic Data Types

Certain characteristics of different types of molecular markers influence the temporal and spatial scales over which they are most informative (Garrick et al. 2010). For example, the lag time between when a lineage divergence event actually occurred and when it registers a genetic signature can be affected by the mutation rate of different genomic regions, as well as the resolution of the assay itself (e.g., ability to distinguish heterozygotes from homozygotes or to identify mutations that are silent rather than only those that are expressed; Avise 2004; Allendorf et al. 2013). Molecular markers also differ in their level of selective constraint and the extent to which they can be connected across unrelated studies (Caterino et al. 2000; Sunnucks 2000). To date, population-level studies of saproxylic insects have employed several different types of molecular markers. Direct sequencing of mtDNA—particularly the cytochrome oxidase I (COI) gene region—has been one of the most common approaches (Table 25.1). Mitochondrial sequence data can be readily generated for diverse insect taxa due to the availability of broadly useful polymerase chain reaction (PCR) primers (e.g., Folmer et al. 1994; Simon et al. 1994). Furthermore, mtDNA sequences are phylogenetically informative. One shortcoming, however, is that the entire mitochondrial genome is effectively a single locus, and so when mtDNA data are used alone, there is little scope for cross-validation of inferences (Edwards and Beerli 2000). Nuclear microsatellite markers

Table 25.1 Summary of genetic data types, and number of individuals sampled and screened for genetic variation, in population-level studies of saproxylic insects

Region/taxon	Common name	IUCN status	Continent or country	Genetic data type and no. of loci (no. of individuals)						References
				Mt. seq.	Indel	Microsat.	Allozyme	AFLP	RAPD	
Northern Hemisphere										
Blattodea										
Cryptocercus punctulatus	Wood roach	–	E. USA	1 (95)	–	–	–	–	–	Garrick et al. (2017)
Reticulitermes flavipes	Subterranean termite	–	E. USA and W. Europe	1 (215)	–	12 (170)	–	–	–	Perdereau et al. (2013)
Reticulitermes grassei	Subterranean termite	–	SW. France	1 (52)	–	6 (512)	–	–	–	Bankhead-Dronnet et al. (2015)
Zootermopsis angusticollis	Damp-wood termite	–	W. USA	–	–	9 (963)	–	–	–	Booth et al. (2012)
Zootermopsis nevadensis	Damp-wood termite	–	W. USA	–	–	12 (468–972)	–	–	–	Aldrich and Kambhampati (2007)
Coleoptera										
Bolitophagus cornutus[a]	Fungus beetle	–	E. USA	–	–	–	5 (?)	–	–	Whitlock (1992)
Bolitophagus reticulatus[a]	Fungus beetle	–	N. Europe	–	–	–	8 (163)	–	5 (149)	Jonsson et al. (2003)
Cerambyx cerdo	Great capricorn beetle	VU	E. Europe	1 (82)	–	9 (79)	–	–	–	Drag and Cizek (2015)
Cucujus cinnaberinus	Flat bark beetle	NT	N. and E. Europe	–	–	10 (71)	–	–	–	Røed et al. (2014)
Diaperis boleti[a]	Fungus beetle	–	E. Europe	–	–	–	–	81 (136)	–	Oleksa (2014)

(continued)

Table 25.1 (continued)

Region/taxon	Common name	IUCN status	Continent or country	Genetic data type and no. of loci (no. of individuals)						References
				Mt. seq.	Indel	Microsat.	Allozyme	AFLP	RAPD	
Elater ferrugineus	Red click beetle	[b]	E. Europe	–	–	–	–	105 (247)	–	Oleksa et al. (2015)
Oplocephala haemorrhoidalis[a]	Fungus beetle	[b]	N. Europe	–	–	–	10 (83)	–	26 (101)	Jonsson et al. (2003)
Osmoderma barnabita	Hermit beetle	NT	E. Europe	–	–	–	–	91 (99)	–	Oleksa et al. (2013)
Protaetia marmorata	Marbled rose chafer	–	E. Europe	–	–	–	–	89 (136)	–	Oleksa et al. (2013)
Pytho abieticola	Beetle	–	N. and E. Europe, China	1 (39)	–	–	–	–	–	Painter et al. (2007)
Pytho depressus	Beetle	–	N. and E. Europe, China	1 (97)	–	–	–	–	–	Painter et al. (2007)
Pytho kolwensis	Beetle	–	N. and E. Europe, China	1 (145)	–	–	–	–	–	Painter et al. (2007)
Rosalia alpina	*Rosalia* longhorn beetle	VU	Europe	1 (164)	–	8 (695)	–	–	–	Drag et al. (2015)
Diptera										
Blera fallax	Pine hoverfly	[b]	N. Europe	–	–	12 (72)	–	–	–	Rotheray et al. (2012b)

Southern Hemisphere										
Blattodea										
Mastotermes darwiniensis	Giant northern termite	–	N. Australia	–	–	6 (1591)	–	–	–	Goodisman and Crozier (2002)
Microhodotermes viator	Southern harvester termite	–	W. South Africa	–	–	7(369)	–	–	–	Muna and O'Ryan (2016)
Coleoptera										
Agyrtodes labralis[a]	Fungus beetle	–	S. New Zealand	1 (187)	–	–	–	–	–	Marske et al. (2009)
Adelium calosomoides	Beetle	–	SE. Australia	–	2 (963)	–	5 (963)	–	–	Schmuki et al. (2006b)
Apasis puncticeps	Beetle	–	SE. Australia	–	3 (678)	–	5 (678)	–	–	Schmuki et al. (2006b)
Brachynopus scutellaris[a]	Rove beetle	–	New Zealand	1 (113)	–	–	–	–	–	Leschen et al. (2008)
Epistranus lawsoni[a]	Fungus beetle	–	New Zealand	1 (168)	–	–	–	–	–	Marske et al. (2011)
Pristoderus bakewelli[a]	Fungus beetle	–	New Zealand	1 (88)	–	–	–	–	–	Marske et al. (2011)

Literature searches were conducted using functions in Scopus, an abstract and citation database for peer-reviewed literature. If several papers for a given species were identified, only the paper with most extensive geographic sampling was included in this survey. Conservation status is based on the International Union for Conservation of Nature (IUCN) Red List v.2017-1, with abbreviations of categories as follows: near threatened (NT), vulnerable (VU), or not assessed (–). Other abbreviations are mitochondrial DNA sequence (Mt. seq.), nuclear loci with insertion-deletion mutations (Indel), nuclear microsatellite loci (Microsat.), amplified fragment length polymorphism (AFLP) loci, and randomly amplified polymorphic DNA (RAPD) loci

Continent or country abbreviations are as follows: northern (N.), eastern (E.), southern (S.), western (W.), southeastern (SE.), and southwestern (SW.)

[a]Lives in and/or feeds on fruiting bodies of fungi associated with rotting wood

[b]Characterized as threatened or endangered by author(s)

have also been widely used in intraspecific studies (Table 25.1). These fast-evolving noncoding regions yield information on diploid genotypes of individuals, which are reshuffled each generation in sexually reproducing species (Sunnucks 2000; Garrick et al. 2010). Accordingly, microsatellites can be informative over short timescales and fine spatial scales. However, since microsatellite loci are usually screened using species-specific PCR primers, an initial labor-intensive development and validation phase is required (e.g., Vargo 2000; Goodisman et al. 2001; Aldrich and Kambhampati 2004; Dronnet et al. 2004 Runciman et al. 2006; Rotheray et al. 2012a; Drag et al. 2013a, b; Røed et al. 2014; Yaguchi et al. 2017).

Compared to mtDNA sequencing and nuclear microsatellite genotyping of saproxylic insects, allozyme (i.e., protein electrophoresis) assays have been less frequently used (Table 25.1). Although these markers provide information on diploid genotypes of individuals and have the benefit of being attainable for diverse insect taxa, allozyme loci typically exhibit low polymorphism and thus provide poor resolution. Also, technical issues such as the need for fresh tissue stored on ice limit the utility of these markers. Two other types of molecular data that have been applied in population-level studies of saproxylic insects are amplified fragment length polymorphism and randomly amplified polymorphic DNA loci. In both cases, numerous anonymous loci, presumably with a genome-wide distribution, are simultaneously amplified via PCR to provide individual-based DNA profiles. While these profiles can be analyzed on the basis of shared versus non-shared bands following separation by size on an electrophoretic gel, the inability to distinguish heterozygotes from homozygotes and the potential for the lack of homology among fragments of the same size can complicate interpretation (Sunnucks 2000). Finally, screening of nuclear insertion-deletion mutations has also occasionally been used (Table 25.1; also see Runciman et al. 2006; Schmuki et al. 2006a). As with microsatellites, these markers can be informative over fine spatial scales but also often require extensive development of and testing of PCR primers. High-throughput screening of single nucleotide polymorphisms—an emerging data type that makes use of next-generation sequencing platforms—has not yet been applied to saproxylic insect population genetics. However, Dillard (2017) successfully used single nucleotide polymorphisms for paternity analysis of the wood-feeding horned passalus beetle, *Odontotaenius disjunctus* (Illiger). Thus, issues relating to the lack of resolution may soon be overcome by new approaches.

25.3.2 Overview of Research Questions and Findings

Although the goals of intraspecific assessments of diversity in saproxylic insects have been broad, a number of recurring themes are apparent. For example, population-level studies of eusocial insects such as termites have often used genetic data to understand colony structure. Specifically, investigations have focused on demarcating colony boundaries, distinguishing between simple and extended family colonies (i.e., a single pair of unrelated alate-derived reproductives versus many

full-sib neotenic reproductives), characterizing the relationship between geographic distance and relatedness, and partitioning of genetic variation across different spatial scales (e.g., individuals, colonies, forest regions; Goodisman and Crozier 2002; Aldrich and Kambhampati 2007; Booth et al. 2012; Perdereau et al. 2013; Bankhead-Dronnet et al. 2015; Muna and O'Ryan 2016). Conversely, population-level studies of threatened or endangered saproxylic insects have typically focused on quantifying levels of genetic diversity and estimating the effective number of breeding individuals within local populations. Conservation-oriented studies have also assessed evidence for inbreeding and/or past bottlenecks and determined the magnitude of gene flow limitation among populations—often in the context of habitat fragmentation or other potential dispersal barriers (Jonsson et al. 2003; Rotheray et al. 2012b; Oleksa et al. 2013, 2015; Røed et al. 2014; Drag and Cizek 2015; Drag et al. 2015). Additionally, researchers have used landscape genetic analyses to understand the permeability of different habitat types to dispersal of individuals (Schmuki et al. 2006b; Oleksa et al. 2015). Finally, some studies of saproxylic insects have focused on reconstructing historical events that generated high intraspecific genetic diversity, such as climatically driven lineage splitting followed by long-term isolation of populations in separate refuges (Painter et al. 2007; Leschen et al. 2008; Marske et al. 2009, 2011; Drag et al. 2015; Garrick et al. 2017).

Literature survey data (Table 25.2) showed that most population-level studies of saproxylic insects have been conducted over relatively large spatial scales (i.e., >200 km between the most distant sites). Considering that dispersal abilities of these organisms are often presumed to be very limited (e.g., Ranius and Hedin 2001), it is not surprising that marked genetic structure has repeatedly been detected. Interestingly, the manner in which the basic units used for analyses are defined seems to impact the number of different populations that are reported to exist within a given species. In general, compared to objective criteria that consider only natural genetic groups that are detected via clustering analyses, the use of more subjective criteria (e.g., number of collection sites) tends to result in more populations being recognized (Table 25.2). Whether this discrepancy represents insensitivity in the clustering analyses and/or upward bias in the investigator-reliant approach remains unclear. However, in the interest of promoting standardized methods that facilitate comparisons among studies, routine reporting of the number of natural genetic clusters would be beneficial.

Estimated levels of within-population diversity and between-population differentiation can be strongly impacted by genetic data type (Avise 2004). Our literature survey showed that studies that used allozyme loci and/or anonymous genetic markers such as amplified fragment length polymorphisms (Jonsson et al. 2003; Schmuki et al. 2006b; Oleksa et al. 2013, 2015; Oleksa 2014; Table 25.1) reported the lowest values of expected heterozygosity and the fixation index F_{ST}, respectively (Table 25.2). However, within genetic data type classes, comparisons across studies are possible, such that basic trends should be identifiable. For mtDNA sequence datasets, levels of within-population diversity were moderate to high, with values of haplotypic diversity (i.e., the probability that two randomly chosen sequences are

Table 25.2 Summary of spatial scale of sampling, population genetic structure, and levels of genetic diversity and differentiation, in population-level studies of saproxylic insects

Region/taxon	Sampling scale (km)	No. of different populations			Intrapopulation diversity		Inter-population differentiation		References
		Defined a priori	Defined a posteriori						
			Mt. seq.	Other data types	Mt. seq. (Hd)	Other data types (He)	Mt. seq. (F_{ST})	Other data types (F_{ST})	
Northern Hemisphere									
Blattodea									
Cryptocercus punctulatus	880	–	5	–	NR	–	NR	–	Garrick et al. (2017)
Reticulitermes flavipes	>1000	–	2	4 or 7	NR	Very high (0.71)	NR	NR	Perdereau et al. (2013)
Reticulitermes grassei	440	–	1	2 or 3	NR	NR	NR	Moderate (0.14)	Bankhead-Dronnet et al. (2015)
Zootermopsis angusticollis	150	–	–	3	–	Moderate (0.31)	–	Very high (0.43)	Booth et al. (2012)
Zootermopsis nevadensis	320	2[b]	–	–	–	High (0.44)	–	NR	Aldrich and Kambhampati (2007)
Coleoptera									
Bolitophagus cornutus[a]	5	2	–	–	–	–	–	–	Whitlock (1992)
Bolitophagus reticulatus[a]	>1000	10	–	–	–	Very low (0.08)	–	Very low (0.04)	Jonsson et al. (2003)
Cerambyx cerdo	175	–	1	2	Moderate (0.37)	Very high (0.57)	Low (0.08)	Low (0.06)	Drag and Cizek (2015)
Cucujus cinnaberinus	>1000	2	–	–	–	High (0.48)	–	NR	Røed et al. (2014)
Diaperis boleti[a]	230	15	–	–	–	Moderate (0.35)	–	Very low (0.03)	Oleksa (2014)

Elater ferrugineus	18	10	–	–	–	Moderate (0.29)	–	Low (0.07)	Oleksa et al. (2015)
Oplocephala haemorrhoidalis[a]	>1000	6	–	–	–	Very low (0.07)	–	Moderate (0.12–0.27)	Jonsson et al. (2003)
Osmoderma barnabita	200	7	–	–	–	Low (0.21)	–	Moderate (0.11)	Oleksa et al. (2013)
Protaetia marmorata	200	11	–	–	–	Moderate (0.29)	–	Very low (0.03)	Oleksa et al. (2013)
Pytho abieticola	>1000	–	2	–	Very high (0.92)	–	High (0.61)	–	Painter et al. (2007)
Pytho depressus	>1000	–	2	–	Very high (0.92)	–	Moderate (0.42)	–	Painter et al. (2007)
Pytho kolwensis	>1000	–	2	–	High (0.58)	–	Moderate (0.49)	–	Painter et al. (2007)
Rosalia alpina	>1000	–	1 or 2	2	Moderate (0.38)	High (0.45)	Low (0.12)	Moderate (0.12)	Drag et al. (2015)
Diptera									
Blera fallax	>1000	–	–	2	–	Moderate (0.4)	–	(Moderate (0.13)	Rotheray et al. (2012b)
Southern Hemisphere									
Blattodea									
Mastotermes darwiniensis	>1000	20	–	–	–	NR	–	NR	Goodisman and Crozier (2002)
Microhodotermes viator	80	4	–	–	–	Moderate (0.42)	–	Moderate (0.20)	Muna and O'Ryan (2016)
Coleoptera									
Agyrtodes labralis[a]	780	–	6 or 7	–	NR	–	NR		Marske et al. (2009)

(continued)

Table 25.2 (continued)

Region/taxon	Sampling scale (km)	No. of different populations			Intrapopulation diversity		Inter-population differentiation		References
		Defined a priori	Defined a posteriori						
			Mt. seq.	Other data types	Mt. seq. (Hd)	Other data types (He)	Mt. seq. (F_{ST})	Other data types (F_{ST})	
Adelium calosomoides	10	NR	–	–	–	NR	–	NR	Schmuki et al. (2006b)
Apasis puncticeps	10	NR	–	–	–	NR	–	NR	Schmuki et al. (2006b)
Brachynopus scutellaris[a]	730	–	4	–	NR	–	NR	–	Leschen et al. (2008)
Epistranus lawsoni[a]	>1000	–	4	–	NR	–	NR	–	Marske et al. (2011)
Pristoderus bakewelli[a]	>1000	–	6	–	NR	–	NR	–	Marske et al. (2011)

The literature search and papers cited follows Table 25.1. Sampling scale is the maximum distance between a pair collection sites. Populations identified a priori are those that were defined by collection sites alone (or via other subjective grouping schemes). Conversely, populations identified a posteriori each form natural groups within the empirical genetic datasets (e.g., clades on a phylogenetic tree or panmictic genotypic clusters). Inferences based on mtDNA sequences (Mt. seq.) are distinguished from those based on any of the other genetic data types listed in Table 25.1. Standardized measures of intrapopulation diversity are haplotypic diversity (Hd; the probability that two randomly chosen sequences are different) and expected heterozygosity (He; the proportion of individuals that will be heterozygous at a locus assuming Hardy-Weinberg equilibrium); if these were calculated from multiple populations and loci, the mean value is reported. Across surveyed studies, the most commonly used metric of inter-population differentiation was F_{ST} (a measure of allele frequency differences). "NR" indicates data were not reported

[a]Lives in and/or feeds on fruiting bodies of fungi associated with rotting wood

[b]Classified as different subspecies

different) ranging from 0.37 to 0.92 when averaged across each population in a given study. Surprisingly, however, mtDNA-based population differentiation was generally moderate to low. Microsatellite data also tended to show moderate to very high diversity within populations, but unlike mtDNA, population differentiation was seldom low (Table 25.2). This may reflect inherent differences in the spatial scale of resolution among marker types. Although only two studies in our survey employed both mtDNA and microsatellite data and reported the standard diversity statistics that we tracked (i.e., Drag and Cizek 2015; Drag et al. 2015; Table 25.1), both showed reasonable consistency between data types in terms of inferences about levels of diversity and differentiation (Table 25.2).

25.4 Genus-Level Diversity and Integrative Taxonomy

25.4.1 Genetic Data Types

Genus-level studies of saproxylic insects have mostly used mtDNA sequence data, but the gene region(s) targeted varies by taxonomic group. Generally speaking, whereas termite studies have tended to focus on the 16S ribosomal RNA gene, beetle studies have almost exclusively used the COI gene (Table 25.3). Notably, for some beetle groups, nuclear DNA regions have been sequenced in conjunction with mtDNA. In these cases, protein-coding regions (e.g., *wingless*) or non-coding regions (e.g., internal transcribed spacer) have been used (Table 25.3). In most cases, however, authors have reported that nuclear DNA sequence datasets were less informative than corresponding mtDNA datasets, owing to fewer variable nucleotide positions in multi-sequence alignment. In addition to direct sequencing, some genus-level studies have evaluated the utility of cost- and time-efficient assays for screening known DNA sequence variants. These approaches have included restriction fragment length polymorphism (RFLP) assays, as well as modifications of PCR primers so that successful amplification occurs only for a given species (e.g., species-specific and multiplex PCR methods; Table 25.3).

25.4.2 Overview of Research Questions and Findings

Goals of genus-level applications of molecular data to saproxylic insects fall into three major categories: phylogenetic relationships across the tree of life, rapid species identification, and reassessment of existing taxonomy (Timmermans et al. 2010). Mitochondrial DNA barcode sequences have been effective for reconstructing some phylogenetic relationships (Timmermans and Vogler 2012). However, COI is not a universally appropriate gene for estimating relationships for every taxon. For example, rapid radiations present a challenge because incomplete lineage sorting is prevalent, whereas high levels of homoplasy (i.e., repeated

Table 25.3 Summary of molecular approaches, taxon sampling, and goals/findings of genus-level studies of saproxylic insects

Region/taxon	Common name	Continent or country	Molecular assay	Gene region(s)	No. of focal taxa	Goal	Major conclusion	References
Blattodea								
Cryptocercus spp.	Wood roaches	USA	Sequencing	mtDNA 12S and 16S rRNA	1	Assess taxonomy	At least two spp. in the USA (one new)	Kambhampati et al. (1996)
		E. USA	Sequencing	mtDNA 12S and 16S rRNA	1	Assess taxonomy	Four spp. in E. USA (three newly named)	Burnside et al. (1999)
Reticulitermes spp.	Subterranean termites	S. USA	Sequencing	mtDNA COII	3	Spp. identification	At least one cryptic sp. likely to exist	Jenkins et al. (2000)
		C. USA	Sequencing	mtDNA 16S rRNA	4	Spp. distributions	Spp. patchily distrib-uted in Texas	Austin et al. (2004)
		S. and C. USA	Sequencing	mtDNA D-loop	3	Spp. identification	Assay accurate for at least one sp.	Foster et al. (2004)
		Americas and Europe	Sequencing	mtDNA 16S rRNA	2	Assess taxonomy	Two named spp. synonymized	Austin et al. (2005)
		W. USA	Sequencing	mtDNA COII	2	Assess taxonomy	Several cryptic spp. likely to exist	Copren et al. (2005)
		C. USA	Sequencing	mtDNA 16S rRNA	4	Spp. distributions	Known range extended for one sp.	Austin et al. (2006)
		E. USA	Sequencing	mtDNA 16S rRNA	6	Assess taxonomy	Recently described sp. validated	Austin et al. (2007)
		E. USA	Sequencing	mtDNA 16S rRNA	3	Spp. identification	Assay accurate for at least two spp.	King et al. (2007)
		E. USA	Sequencing	mtDNA COI and COII	5	Assess taxonomy	1 new sp. described and named	Lim and Forschler (2012)
		E. USA	PCR-RFLP	mtDNA COII	5	Spp. identification	Assay is efficient and accurate	Garrick et al. (2015)

Coptotermes spp.	Subterranean termites	Global	Sp.-specific PCR	mtDNA 16S rRNA	12	Spp. identification	Assay is efficient and accurate	Szalanski et al. (2004)
		Global	Multiplex PCR	mtDNA 16S rRNA	8[a]	Spp. identification	Assay is efficient and accurate	Janowiecki and Szalanski (2015)
		Americas	Sequencing	mtDNA 16S rRNA	6	Assess taxonomy	Three named spp. synonymized	Scheffrahn et al. (2015)
Coleoptera								
Glischrochilus spp.	Sap beetles	N. Europe	Sequencing	mtDNA COI	3	Assess taxonomy	One new sp. described and named	Clayhills et al. (2016)
Lucanus spp.	Giant stag beetles	Europe and Asia	Sequencing	mtDNA COI and nDNA Wg	16	Assess taxonomy	Sp.-level rank valid for threatened sp.	Lin et al. (2011)
		Europe and Asia	Sequencing	mtDNA COI	6	Spp. identification	Assay has mixed success	Cox et al. (2013)
		Italy	Sequencing	mtDNA COI and nDNA Wg	2	Spp. identification	COI assay has fairly good success	Solano et al. (2016)
Osmoderma spp.	European hermit beetles	Europe	Sequencing	mtDNA COI	5	Assess taxonomy	Sp.-level rank valid for named taxa	Audisio et al. (2009)
		N. and E. Europe	Sequencing	mtDNA COI	2	Spp. identification	Confirmed specimen assignments	Svensson et al. (2009)
		N. Europe	Sequencing	mtDNA COI	5	Spp. identification	Finland samples assigned to sp.	Landvik et al. (2013)
		Italy	Sequencing	mtDNA COI	2	Assess taxonomy	Sp.-level rank valid for named taxa	Zauli et al. (2016)
Morimus spp.	Longhorn beetles	Europe and Asia	Sequencing	mtDNA COI and nDNA ITS	5	Assess taxonomy	Five named spp. probably synonyms	Solano et al. (2013)

The literature search excluded strictly phylogenetic studies (i.e., those focused on resolving relationships among named species and estimating divergence dates). Note that this table only presents data from representative exemplars, as that some groups (e.g., pest species such as termites) have been extensively studied using molecular data for decades. Abbreviations are as follows: polymerase chain reaction (PCR); mitochondrial DNA (mtDNA); ribosomal RNA (rRNA); nuclear DNA (nDNA); and species (sp. = singular, spp. = plural)

Continent or country abbreviations are as follows: northern (N.), eastern (E.), southern (S.), western (W.), and central (C.)

[a]Relates to congeneric taxa only

mutations at same site, leading to saturation) become problematic for deeper-level relationships. Accordingly, for research focused on resolving phylogenetic relationships at the genus-level or higher and estimating divergence times among lineages, multiple independent loci are often needed.

The published research associated with rapid species identification can be divided into molecular toolset development versus application, where the latter includes investigations that seek to better understand species' geographic distributions (Table 25.3). Interestingly, whereas assays such as PCR-RFLP, species-specific PCR, and multiplex PCR have shown high accuracy, direct sequencing has had mixed success (Table 25.3). However, rather than indicating weaknesses of the latter data type, this probably reflects differences in suitability of the chosen DNA region or taxonomic complexity of the group at hand. Indeed, whereas PCR-RFLP, species-specific PCR, and multiplex PCR are limited by the fact that as-yet unknown variants can complicate interpretation and/or reduce accuracy, direct sequencing coupled with phylogenetic analyses is well-suited to handling newly discovered genetic variants. Indeed, for saproxylic beetles in particular, COI barcodes have shown low rates of species misidentification (Hendrich et al. 2015; Jordal and Kambestad 2014; Pentinsaari et al. 2014; Rougerie et al. 2015a).

In the context of taxonomic reassessments, molecular data have provided several valuable insights. For example, they have clarified situations where two or more named species were suspected to be synonyms (e.g., *Reticulitermes flavipes* (Kollar) and *R. santonensis* Feytaud termites; Table 25.3). Similarly, DNA sequence data also suggested that two European longhorn beetle species, *Anastrangalia dubia* (Scopoli) and *A. reyi* (Heyden), are probably synonyms (Hendrich et al. 2015; Rougerie et al. 2015a). However, among German beetles, almost 3% of specimens DNA barcoded by Hendrich et al. (2015) have low interspecific distances, yet they do not appear to reflect cases of synonymy. Other explanations for such patterns can include introgression through past or ongoing hybridization or recent divergence. Indeed, Jordal and Kambestad (2014) attributed inconsistencies between mtDNA barcodes and morphology-based identification of bark beetles to past hybridization between *Pityophthorus micrographus* L. and *P. pityographus* Ratzeburg. Furthermore, in a study of western Palaearctic stag beetles (Cox et al. 2013), COI could discriminate several named *Lucanus* species and *L. cervus* L. subspecies, but not all could discriminated. Here, haplotype sharing among taxa was suspected to be due to recurrent hybridization events or incomplete lineage sorting. Where mtDNA barcodes and existing taxonomy are discordant, large numbers of individuals from each putative group are usually needed to identify the underlying causes, yet this requirement can be a limiting factor when working with rare or difficult to sample organisms such as saproxylic insects.

In contrast to low interspecific divergences, in some studies, a single named taxon has been shown to exhibit very high levels of genetic diversity (e.g., *Cryptocercus punctulatus* Scudder wood roaches and *Osmoderma eremita* (Scopoli) hermit beetles), leading to the formal description and naming of new species (Table 25.3). Despite a long history of intensive taxonomic research, Pentinsaari et al. (2014) and Hendrich et al. (2015) reported that almost 6% of the North European beetle species

and 7% of the Bavarian beetle species, respectively, contained two or more distinct barcode clusters. Even among the well-known bark beetle species, Jordal and Kambestad (2014) detected the occurrence of a cryptic species of *Dryocoetes*, on the basis of inconsistencies between mtDNA barcodes and morphological identifications. Similarly, Pentinsaari et al. (2014) used geometric morphometrics in combination with host plant characters to propose the existence of two species of beetles nested within one named taxon, *Agrilus viridis* L. However, here the findings based on mtDNA barcodes were more complex, owing to suspected past hybridization events.

Outcomes of taxonomic reassessments of saproxylic insects can have important legislative ramifications, such as when the species-level status of a threatened or endangered species is brought into question (Lin et al. 2009). That said, explicit statements about which species concept is being applied, and criteria used to assess whether empirical data support species-level designation, are critical elements of such studies. At present, the most popular approach involves the use of sequence divergence threshold values (Meier et al. 2006). However, evidence for the existence of a "barcoding gap" (i.e., substantially higher sequence divergence among species cf. within species) should be considered in the context of sampling density, given that diagnosability of related species may diminish as additional specimens are added to the sequence dataset. Also, while DNA taxonomy may be seen as a practice of its own, some researchers suggest that its most valuable role lies in providing systematists a first approximation to delimit taxa and rapidly assess species number (Janzen et al. 2009; Lamarre et al. 2016). Barcoding may also be used as an exploratory tool, revealing cases needing further investigation. Fortunately, analytical developments are facilitating the use of DNA sequence data in species delimitation (e.g., Yang and Rannala 2010; Ence and Carstens 2011). Although the newer approaches are not without caveats (Carstens et al. 2013; Sukumaran and Knowles 2017), these data-driven assessments provide working hypotheses for focused follow-up work. Given that the genes used in molecular taxonomy may not be functionally correlated with speciation, integrative taxonomy should embrace all available evidence (e.g., adult and larval morphology including color and pattern where relevant, molecular data, behavioral characters including mating displays and/or phenology, as well as ecology; Will et al. 2005; Astrin et al. 2012).

25.5 Community-Level Diversity

25.5.1 Genetic Data Types

DNA barcoding is commonly used to characterize metazoan biodiversity and has been successfully used to assess biodiversity (Gibson et al. 2014), bypassing shortfalls of other molecular diagnostic methods (Armstrong and Ball 2005). This approach proposes to use information within a single-standard short-gene region common across all taxa and to access that information by DNA sequencing across

species and laboratories (Hebert et al. 2003). It relies on the assumption that sequences in a ~650-bp fragment of COI are more similar among members of the same species than to sequences of any other species. There is a growing literature demonstrating that COI reliably discriminates species-level differences for a diverse set of animals. However, increasing the spatial scale of sampling often reduces its success (Bergsten et al. 2012), and the rate of success of barcoding also varies across insect orders (e.g., Meier et al. 2006; Pentinsaari et al. 2014). Furthermore, the reliability of a COI barcode as species identifier has been debated, given cases of high intraspecific diversity (Moritz and Cicero 2004). Consequently, additional DNA markers are sometimes used to complement COI (e.g., mtDNA 16S ribosomal RNA gene or nuclear DNA loci; Astrin et al. 2012; Dupuis et al. 2012).

25.5.2 Overview of Research Questions and Findings

The new era of DNA data has cascading effects on saproxylic community biology. For example, from a taxonomic diversity perspective, these data provide tools to help with delineating species entities and with developing efficient mass sample identification strategies, whereas from a functional perspective, they shed light on trophic relationships and interaction networks among species. In addition, from a phylogenetic perspective, DNA data allow for computation of distances among species, as well as diversity indices based on tree topology.

The success of a DNA-based species identification system depends on the completeness and the consistency of a barcode reference library (Cristescu 2014). Comprehensive libraries for several focal saproxylic insect groups (e.g., Coleoptera, Isoptera, and Diptera) need to be developed to permit and streamline reliable identification of species. These barcode libraries are being built in collaboration with expert taxonomists using well-curated natural history collections. Data processing pipelines have recently been developed to detect inconsistencies in large DNA barcode datasets, before submitting them to public data repositories like the Barcode of Life Data System (BOLD) or GenBank (Rulik et al. 2017). The sequencing success from collection material identified down to the species-level in some groups (e.g., beetles) is lower than others. Nevertheless, the current developments offer new opportunities to increase throughput, reduce cost, and improve the success rate of sequencing when DNA is limited in quantity or degraded, as is the case for very small invertebrates or when working with material preserved for several years. Although the application of high-throughput sequencing to generate individual-based DNA barcodes was initially limited by short sequence reads as well as the cost and operability of tagging a large number of specimens, these restrictions are now being overcome (Shokralla et al. 2015). Accordingly, the transfer of DNA barcode library construction from routine Sanger sequencing toward the use of this newer technology is becoming feasible.

For saproxylic beetles, DNA barcodes can distinguish species remarkably well (Pentinsaari et al. 2014; Hendrich et al. 2015; Rougerie et al. 2015a). Large numbers

of European saproxylic beetle species have already been barcoded and are publicly available in BOLD as part of national barcoding campaigns carried out in Europe (Hausmann et al. 2013; Huemer et al. 2014; Pentinsaari et al. 2014; Hendrich et al. 2015; Rougerie et al. 2015a). For example, Pentinsaari et al. (2014) performed a comprehensive test of the effectiveness of DNA barcodes as a tool for Scandinavian beetle identification by sequencing the COI region from 1872 species. A high proportion (98.3%) of these species possessed distinctive barcodes, and furthermore, the Barcode Index Number system in BOLD coincided strongly (in 92.1% of all cases) with known species boundaries. Similarly, Jordal and Kambestad (2014) also demonstrated strong congruence between morphology-based identification and sequence clusters for 151 species in 40 genera of bark and ambrosia beetles. Lower identification success rates have been reported for non-exclusively saproxylic insect groups (e.g., Diptera, Meier et al. 2006). In these cases, mismatches were due to considerable overlap between intra- and interspecific genetic divergence. In beetle studies, the few cases of barcode identification failures involved closely related species that are often difficult to identify by morphological characters, and whose species status is controversial, as indicated by high intraspecific genetic variability, low between-species genetic distances, and evidence for introgression/hybridization at contact zones. Even though COI is a highly discriminant marker for many beetles, Jordal and Kambestad (2014) noted that the occurrence of nuclear mitochondrial pseudogenes (NUMTs), detected in 8 out of 151 bark beetle species, demands a stronger focus on data quality assessment in the construction of DNA barcoding databases. NUMTs are indeed a major pitfall in the few cases where they have been prevalent among sequences produced by standard protocols (Haran et al. 2015). That said, close examination of sequence characteristics can reduce error considerably (Song et al. 2008), and high-throughput sequencing should make it easier to detect NUMTs.

25.5.2.1 Metabarcoding

Using Sanger sequencing of single specimens in ecological studies with hundreds of thousands of specimens to be processed is prohibitively costly and time-consuming (Shokralla et al. 2015). Accordingly, the advent of affordable high-throughput sequencing technologies is revolutionizing the field of biomonitoring (Shokralla et al. 2012; Taberlet et al. 2012a). Metabarcoding is a technique that involves high-throughput sequencing from a bulk mixture of DNA from all sampled specimens (Taberlet et al. 2012a; Yu et al. 2012). This approach is much faster and yet can still be as reliable as biodiversity datasets assembled with Sanger sequencing (Ji et al. 2013). Metabarcoding has been used for assessing the diversity in bulk samples of soil animals such as earthworms (Bienert et al. 2012; Pansu et al. 2015), terrestrial arthropods (Yu et al. 2012; Ji et al. 2013; Zhou et al. 2013; Yang et al. 2014), and associated microbiota (Gibson et al. 2014). This technique has also been used in ecological studies to estimate alpha and beta diversity (Yu et al. 2012; Yang et al. 2014). The underlying technology is advancing quickly, with improved efficiency

and resolution (Deagle et al. 2014; Schnell et al. 2015). However, despite the great potential of metabarcoding, few studies have applied this technique for ecological assessment (Aylagas et al. 2014; Pawlowski et al. 2014).

To date, most studies that have applied high-throughput sequencing of DNA recovered from deadwood focus on bacteria (Hoppe et al. 2015) or fungi. In some of the latter cases, assessment of fungal species richness and composition has been based on direct molecular detection of in situ mycelia, often from sawdust and shavings obtained by drilling logs through sapwood and heartwood (Cuadros-Orellana et al. 2013). These recent bacterial and fungal studies were mainly conducted in Palaearctic boreal and temperate forests (Ovaskainen et al. 2010; Rajala et al. 2011, 2012; Kubartova et al. 2012; Ovaskainen et al. 2013; Jang et al. 2015; Ottosson et al. 2015; Runnel et al. 2015; Van der Wal et al. 2015; Yamashita et al. 2015; Baldrian et al. 2016; Hoppe et al. 2016) or, more rarely, in neotropical forests (Purahong et al. 2017; Vaz et al. 2017). Except for Rougerie et al. (2015b), no other metabarcoding study has addressed the sampling of saproxylic insect communities, but some focused on other insect guilds such as belowground arthropods (Cicconardi et al. 2017), grassland/forest-edge arthropods (Morinière et al. 2016), flying insects (Yu et al. 2012), and bees (Tang et al. 2015). Now that metabarcoding of "biodiversity soups" of insect DNA is becoming reliable (Rougerie et al. 2015b), there is considerable scope for advances in understanding the diversity and composition of saproxylic insect communities and, by extension, for identifying environmental predictors of this diversity (e.g., Lindenmayer et al. 2000; Grove 2002b; Woodman et al. 2006).

Using mtDNA metabarcoding, three alternative workflows could be applied to saproxylic insect samples. Workflow 1 involves extraction of pooled insect DNA directly from the preservative solution (Fig. 25.1). For example, it has been demonstrated by Shokralla et al. (2010) and Hajibabaei et al. (2012) that ethanol, commonly used as a preservative medium for trapping and/or storing specimens, contains DNA from stored organisms that can be directly used for downstream amplification and sequencing. Hajibabaei et al. (2012) reported that using "free DNA" from ethanol preservative was effective in providing sequence information for 87% of taxa identified individually from mixture, as compared to 89% in conventional tissue-based DNA extraction methods. Missing taxa were from species with the lowest abundance (e.g., one individual) in the species mixture. This approach does not require the mashing and mixing of all organisms to form homogenized slurry, and consequently does not result in destruction of individual specimens, thereby rendering subsequent morphological analyses possible. The effectiveness of community ethanol-based DNA nonetheless seems to decrease when preservative liquid has been changed in time (Rougerie et al. unpubl. data). In contrast, workflow 2 involves individual-based DNA extraction from voucher specimens and is therefore more time-consuming yet can retain information that ties a particular specimen to a specific mtDNA sequence (Fig. 25.1). Workflow 3 also involves a time-consuming presorting step but streamlines DNA extraction into a single bulk sample; this approach can yield approximately 30% more high score

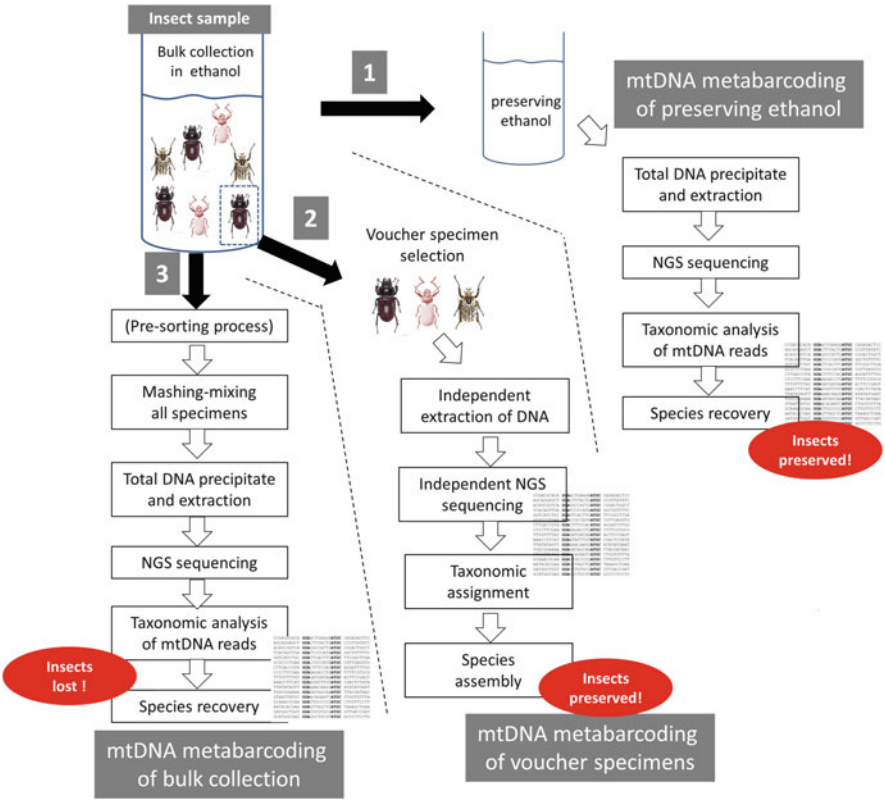

Fig. 25.1 Three alternative workflows for mitochondrial DNA metabarcoding using next-generation sequencing (NGS), each of which could be applied to saproxylic insect bulk collections. Workflows 1 and 2 preserve insect specimens, thereby rendering subsequent morphological study possible, whereas workflow does not

barcode index numbers compared to a non-sorted sample (Morinière et al. 2016; Fig. 25.1).

Once DNA has been extracted, PCRs are usually performed in several replicates and at different annealing temperatures to diminish amplification bias, and products are pooled before sequencing to correct for within-sample variation (Zhan et al. 2014) and to avoid spurious overestimation of operational taxonomic unit (OTU) diversity. The use of Illumina MiSeq or HiSeq sequencing platforms ensures that sufficient read depth is obtained for the detection of rare species in the samples. For each sample, the DNA is tagged with specific sequence identifiers, to ensure the traceability of the OTUs and species identification from the sequences back to the collecting event (or individual specimens, depending on the workflow used; see Fig. 25.1). The development of bioinformatics pipelines to determine OTUs from high-throughput sequence data is a rapidly advancing field, and existing tools are becoming more efficient (Yu et al. 2012). Most pipelines involve removal adaptor

sequences and low-quality reads, followed by the assembly of high-quality sequences, and then the assignment of sequences to different OTUs based on overall sequence similarity. However, OTU delimitation can differ among taxa (Fontaneto et al. 2015). Several clustering methods (Zhang et al. 2013) exist to infer the taxa present in the samples in an effort to unify the definition of OTUs and streamline analyses. The RESL approach in BOLD (Ratnasingham and Hebert 2013) has been effective in objectively delimiting the OTUs and assigning a species name through sequence matching to barcode index numbers in saproxylic beetles (Pentinsaari et al. 2014; Rougerie et al. 2015a).

25.5.2.2 Community Diversity and Structure

Pipelines that use high-throughput DNA sequencing generate molecular operational taxonomic units (MOTUs), based on pairwise distance and a user-defined sequence divergence cutoff. MOTU-based metrics are useful species surrogates to describe community richness. From these data, information on evolutionary relationships among MOTUs is easily attainable. The value of phylogenetically based measures of biodiversity has been advocated for some time, but a wider appreciation of their broad utility, including in high-profile study systems, has occurred only recently (e.g., King 2009; Morlon et al. 2011; Frishkoff et al. 2014). Indeed, phylogenetically derived biodiversity metrics are useful for assessing ecosystem functioning (Paquette et al. 2015). One such metric, phylogenetic diversity, quantifies the amount of shared evolutionary history (total branch lengths) among lineages that occur within a location, the context of a tree estimated from all lineages that were sampled across all locations (Faith 1992, 2002). Another metric, phylogenetic endemism, measures the spatial restriction of phylogenetic diversity (Rosauer et al. 2009). Together, these can be viewed as phylogenetic analogs of species richness and turnover, respectively.

In addition to providing opportunities for unbiased measures of local biodiversity in saproxylic arthropods, DNA sequence data may also enable taxonomic assignment of specimens to named species using reference DNA barcode libraries. In such cases, metabarcoding provides accurate measurements of species richness from bulk and environmental samples at an affordable cost. However, one of the limitations relates to the occurrence of natural DNA contaminants, such as sequences derived from prey in gut predatory insects. Another limitation of assessing diversity using metabarcoding is that PCR amplification may cause strong biases, thereby preventing the use of read numbers to estimate the relative abundances of different taxa, and so the technique produces occurrence data only. Recent studies have proposed targeting whole mitochondrial genomes instead of a single or few DNA fragments, and to use shotgun sequencing of bulk or environmental samples, thus bypassing PCR amplifications and inherent biases (i.e., amplification stochasticity, taxon biases, loss of quantitative data; Zhou et al. 2013; Andújar et al. 2015; Gomez-Rodriguez et al. 2015; Tang et al. 2015). This approach would ideally be used in conjunction with the assembly of reference mitogenome libraries for the focal

groups. So far, results of this PCR-free mitogenomic approach have been encouraging for bulk samples with relatively few species and individuals (Andújar et al. 2015; Gómez-Rodríguez et al. 2015).

Measures of changes in functional community structure and food webs require species-level identifications to allow linking species counts to pre-existing databases of functional traits (except in the case of work on intraspecific trait variability; Violle et al. 2012). Whereas the roles of abiotic factors in shaping local forest communities have been well studied, the role of species interactions has received little attention. Most current biomonitoring programs ignore the complex ecological networks of species interactions, which are crucial to take into account if we want to understand the ecological responses of communities to environmental stressors (Gray et al. 2014). Taking tree-insect-parasitoid ecological networks as an illustration, Evans et al. (2016) argued that combining DNA metabarcoding approaches with ecological network analysis presents important new opportunities for understanding large-scale ecological processes. PCR-based molecular gut content analyses may be used to characterize predator-prey or host-parasitoid interactions. Only one PCR-based molecular gut content analysis is known for communities of saproxylic insects (Schoeller et al. 2012), but several studies exist for communities of other insect groups (e.g., Foltan et al. 2005; Eitzinger et al. 2013; Paula et al. 2016). Using gut DNA content screening, Schoeller et al. (2012) characterized interactions between field-collected *Monochamus titillator* (F.) and other wood borers and demonstrated facultative intra-guild predation. Moreover, employing DNA barcoding to identify their morphologically indistinct immature life stages illustrated the power of molecular data to complement and enhance the morphological approach to insect diagnoses. Given the importance of larvae in saproxylic food webs, molecular identification could improve our understanding of saproxylic networks.

25.5.2.3 Molecular Insect Monitoring

There is an increasing need for real-time, large-scale biomonitoring with immediate feedback into management frameworks. The current monitoring programs of forest biodiversity are taxonomically constrained and ill equipped to cover large geographic scales. Traditional biomonitoring schemes are too labor intensive and costly to handle large numbers of specimens, given that they involve examining each individual separately (Lebuhn et al. 2013). In addition, biomonitoring is often biased toward certain taxa, avoiding groups for which taxonomic expertise is unavailable. Invertebrates are rarely used as study groups despite their ecological importance because of their hyperdiversity and the taxonomic impediment (Ebach et al. 2011). Furthermore, traditional biomonitoring schemes often use morphospecies as surrogate of species, thus underestimating actual species numbers, especially in the richest taxa that require careful examination in the laboratory (Derraik et al. 2002). These studies are also unable to account for immature stages in most groups. The combination of emerging genomic technologies and bioinformatics in DNA metabarcoding is strengthening our capacity to process many samples collected at

a large scale for long-term ecological studies that measure the impact of global change on biodiversity. Numerous tools already exist varying in complexity, accuracy, and costs, for biomonitoring marine (e.g., Aylagas et al. 2014) and freshwater ecosystems (Woodward et al. 2013). Biomonitoring pipelines that streamline the identifications of large numbers of specimens and provide accurate, rapid, and cost-efficient measurements of saproxylic insect diversity are needed. Also, few metabarcoding studies to date have focused on groups for which a library was available beforehand. Approaches that can go beyond assigning sequences to MOTUs followed by examination of alpha and beta diversity will bring much more insight into ecological questions. These perspectives strengthen the importance of developing reliable reference databases for species identification.

DNA barcoding allows the rapid and accurate identification of alien and pest species, including morphologically indistinct taxa. It is now widely employed in contexts ranging from monitoring pests (Ashfaq et al. 2016) to supporting the detection of invasive species (Armstrong and Ball 2005). In China for instance, DNA identification of *Xyleborus* species (i.e., ambrosia beetles associated with solid wood-packing materials and very commonly intercepted at ports) has been successfully developed to monitor and prevent invasion (Chang et al. 2014).

High-throughput sequencing allows the detection of an organism following secondary transfer of its DNA to environmental samples. Metagenomic techniques are already in place for preparation of environmental DNA from soil or water (Lodge et al. 2012; Yoccoz et al. 2012; Schmidt et al. 2013; Bohmann et al. 2014). A specifically designed workflow could be developed to treat large volumes of substrate and enable detection of insect larvae in deadwood or in tree-related microhabitats (e.g., wood mold in tree cavities, lignicolous fungus sporocarp). These techniques have already been used to detect deadwood-associated fungi (e.g., Cuadros-Orellana et al. 2013). Several studies have shown promising results for invertebrate species identification from frass (Sint et al. 2015). The analysis of wood samples has the potential to revolutionize forest biomonitoring by allowing foresters to obtain accurate measures of biodiversity, including insects, from dead branches without complex and expensive sampling procedures. The processing of a large volume of substrate and its physical structure may however prove challenging for DNA extraction, although recent results on large volumes of soil (Taberlet et al. 2012b) are encouraging. Crucial steps for wood samples would be the homogenization of large volume of substrate in a grinding mill, and protocol optimization of DNA extraction from wood as secondary compounds, such as terpenoids, might inhibit subsequent PCR amplifications.

25.6 Emerging Directions

Many of the same research questions and molecular approaches highlighted above have been applied to other groups of saproxylic invertebrates [e.g., velvet worms (Sunnucks and Wilson 1999; Trewick 2000; Oliveira et al. 2011; McDonald and

Daniels 2012), terrestrial flatworms and springtails (Alvarez-Presas et al. 2011; Garrick et al. 2012 and references therein), land snails (Hugall et al. 2002), pseudoscorpions (Ranius and Douwes 2002), spiders (Beavis et al. 2011), and millipedes (Walker et al. 2009)]. Accordingly, trends seen in insects may be representative of a broader array of studies that have attempted to understand distributions of diversity in this functionally important ecological community. Indeed, given the taxonomic and geographic breadth of studies published over the past two decades, a broad synthesis of insights from genetics for conservation of saproxylic invertebrates as a whole should now be possible. It is also noteworthy that genomic and transcriptomic tools are increasingly being applied to saproxylic invertebrates [e.g., velvet worms (Roeding et al. 2007), termites (Cameron and Whiting 2007; Zhou et al. 2008; Tartar et al. 2009), wood roaches (Hayashi et al. 2017), and springtails (Wu et al. 2017)]. These genome-wide molecular datasets, coupled with comparative and/or functional analyses, are now enabling previously intractable questions to be addressed.

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