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Resolving Food-Web Structure

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Abstract

Food webs are a major focus and organizing theme of ecology, but the data used to assemble them are deficient. Early debates over food-web data focused on taxonomic resolution and completeness, lack of which had produced spurious inferences. Recent data are widely believed to be much better and are used extensively in theoretical and meta-analytic research on network ecology. Confidence in these data rests on the assumptions (*a*) that empiricists correctly identified consumers and their foods and (*b*) that sampling methods were adequate to detect a near-comprehensive fraction of the trophic interactions between species. Abundant evidence indicates that these assumptions are often invalid, suggesting that most topological food-web data may remain unreliable for inferences about network structure and underlying ecological and evolutionary processes. Morphologically cryptic species are ubiquitous across taxa and regions, and many trophic interactions routinely evade detection by conventional methods. Molecular methods have diagnosed the severity of these problems and are a necessary part of the cure.

1. INTRODUCTION

> Food web ecology has always had to deal with the demons of resolution.
>
> —Smith et al. (2011, p. 9)

A food web is an attempt to describe what the organisms in a community eat—their trophic interactions with other organisms. The study of food webs has many branches, and food webs can be conceptualized and drawn in various ways to derive different kinds of inferences. Accordingly, the term food-web structure can signify various properties. The most literal and common usage refers to a graph depicting species (as nodes/vertices) and their trophic interactions (as links/edges) with other species. The structure of the food web is the topology of this network. This definition is our focus, and we use structure and architecture synonymously with topology throughout, although we acknowledge that there are other valid meanings of food-web structure (e.g., energy pathways, trophic pyramids, food-chain lengths).

By studying food webs and their structure, researchers seek to answer a range of questions that is almost as broad as ecology itself. Indeed, it has been argued that food webs provide a unifying framework for linking the conventional subdisciplines of ecology (Thompson et al. 2012). Through the lens of food webs, ecologists investigate energy flows and nutrient transfers, population dynamics, indirect effects, ecological niches and competitive interactions, species coexistence and biodiversity, specialization and coevolution, ecosystem functions, the stability of populations and communities, the robustness of systems to environmental changes, and various other phenomena (Moore et al. 2018). Many studies distinguish different categories of food webs, such as those involving insect hosts and their parasitoids and those consisting primarily of mutualistic interactions between plants and their pollinators, protectors, and seed dispersers (Ings et al. 2009). Here, we refer to food webs inclusively to describe ecological networks of consumer-resource interactions. We believe that our observations and arguments pertain equally to all such networks.

When Smith et al. (2011, p. 9) called out "the demons of resolution," they were referring to a problem that has dogged food-web ecology since its inception. Gathering the empirical data necessary to assemble and meaningfully study a food web of wild organisms—answering the question, what do animals actually eat?—is enormously difficult for multiple reasons. Researchers have tried to simplify this problem in various ways, often by lumping species into groups thought to share similar characteristics or by proceeding with analyses of data that lack some substantial fraction of the species (nodes) and interactions (links) actually present in an assemblage. Such aggregated or fragmentary data have low resolution—they are blurry; the details are not visible. The resolution of food-web data is demonic because it can radically change network topology and associated biological inferences in ways that are unknowable in the absence of better data. The history of food-web ecology is marked by debates over whether the resolution of the data is adequate to support the conclusions drawn.

Many important questions about food webs can be addressed without high-resolution data on network topology. Examples of significant progress include greater understanding of how top-down control and trophic cascades regulate species' abundances (Power 1990, Terborgh & Estes 2013), of the ways in which food webs are coupled in space (Rooney et al. 2006), of the potency of indirect and trait-mediated effects (Schmitz et al. 2004), and of the factors that determine food-chain length (Post 2002). Not coincidentally, these advances have been fueled by the interplay of testable theory, observational data, and manipulative experiments. The study of food webs as networks, by contrast, is theoretically and computationally sophisticated (Delmas et al. 2019) but

arguably has not yet produced fundamental advances in our understanding of the biology, in large part because the available empirical data are simply inadequate to enable rigorous tests of mathematical predictions or to expose the mechanisms underlying recurring statistical patterns.

We develop this argument below. We contend that the data sets used to analyze food-web structure are in general much worse than most users appreciate. Recent research in molecular ecology has cast serious doubt on things that ecologists would like to take for granted, such as the ability to correctly delineate species in the field. Related work has shown that things long considered manageably difficult, such as the ability to compile an accurate and quasi-comprehensive list of an animal's foods, are actually extremely difficult, and that many existing lists are far more incomplete and incorrect than commonly assumed. However, we also argue that the same molecular methods that have brought the depth of these problems to light can play an important role in solving them. The title of our article has two intended meanings. One highlights the importance of resolving food-web data—of bringing the details into focus to the finest measurable grain. The other meaning of the word resolve is to bring closure to a dispute or contentious matter. In this regard, early debates about the quality and resolution of food-web data remain relevant, and we briefly review their history.

2. FOOD-WEB STRUCTURE: A BRIEF HISTORY OF IGNORANCE

2.1. A First Wave of Graph Theory

> These qualitative descriptions were never intended to be data, to serve as grist for the theoretician's mill.
>
> —Paine (1988, p. 1652)

Quantitative food-web analysis began trending in the late 1970s, following May's (1973) explorations of the complexity-stability relationship and Cohen's (1978) explorations of the statistical properties of "real food webs" combed from the ecological literature. Subsequent studies, drawing on an incrementally increasing number of published webs, addressed questions that remain open today. Are there universal regularities of food-web organization (Cohen & Briand 1984)? What determines food-web stability (De Angelis 1975) and sensitivity to species loss (Pimm 1980)? What is the dimensionality of dietary niche space, and how much do niches overlap (Cohen 1978)? Are food webs divided into compartments, and what does that mean (Pimm & Lawton 1980)? Is there a consistent relationship between the numbers of predators and prey within communities (Briand & Cohen 1984)? How long are food chains, and why (Pimm & Lawton 1977)? It briefly seemed that food webs would prove "orderly and intelligible" (Pimm et al. 1991, p. 669).

By the mid-1990s, a string of empirical critiques had crushed this optimism (Paine 1988, Polis 1991, Hall & Raffaelli 1993). The supposedly real food webs were grossly incomplete and comically oversimplified. Resolution was uneven and biased; primary producers and small consumers were typically aggregated into broad lumps (e.g., "algae," "land vegetation," "insects," "fish"), whereas mammals and birds were often resolved as species (e.g., "pig," "rat," "man," "boobies") (Cohen 1989, figure 13.1). To standardize such webs, investigators combined organisms that allegedly shared identical sets of foods and predators into "trophic species" (Cohen 1989, p. 183). Subsequent studies showed that putative generalities about food-web structure were sensitive to the taxonomic resolution of the data and to the various contrivances of the analyses (Martinez 1993, Thompson & Townsend 2000, Winemiller 2007). Ecologists were asked to supply better data (Cohen et al. 1993). When some did, it became clear that even moderately resolved whole-community food webs were analytically intractable and contained many assumption-defying

annoyances such as cannibalism, loops, and omnivory (Polis 1991). For a time, food-web ecology turned elsewhere (Polis & Winemiller 1996, Polis et al. 2004).

Bipartite network: a graph in which all links connect two separate groups of nodes (for example, interactions between herbivore species and plant species)

Modularity: the extent to which a web is organized into compartments of species that have many interactions with each other but few with species outside their compartment

Nestedness: the extent to which specialists interact only with subsets of the species that generalists interact with

Rewiring: the reorganization of food-web links that occurs when animals change their diets in response to some perturbation, such as the disappearance of a preferred food

2.2. A Second Wave of Graph Theory: Network Ecology

> Nestedness is an interesting abstract network property that undoubtedly influences the statistical behavior of large systems of differential equations.
>
> —James et al. (2013, p. E3)

Interest in networks accelerated at the turn of the millennium, stimulated by the growth of the internet and enabled by increasing computational power (Strogatz 2001). These developments rejuvenated the study of topological patterns in trophic networks (Dunne et al. 2002, Bascompte et al. 2003, Pascual & Dunne 2006). The core questions have not changed fundamentally and resemble those of community ecology at large, although variations and elaborations on these themes have proliferated—sometimes to the point of getting lost within themselves [what does stability even mean? (Domínguez-García et al. 2019)]. The methods and vocabulary, however, have changed radically. Bipartite networks of predominantly mutualistic trophic interactions—ignored in early food-web theory—have been a particularly salient focus (Bascompte & Jordano 2014). The aims and claims of modern network ecology are reviewed extensively elsewhere (Ings et al. 2009, Delmas et al. 2019), as is the multitude of metrics used to quantify various topological properties (Lau et al. 2017).

Network ecology has produced exciting theoretical developments, but once again, data are limiting. Model predictions are generated and debated, and then they hang, both theoretically and empirically unresolved. Modularity and/or nestedness might promote diversity and/or stability, or they might not (Bastolla et al. 2009, Thébault & Fontaine 2010, Allesina & Tang 2012, James et al. 2012, Grilli et al. 2016). Behavioral rewiring of species interactions might buffer communities against extinction (Valdovinos et al. 2010) or not (Gilljam et al. 2015). Metrics abound, but it is unclear "which of the many available measures actually hold ecological meaning" (Delmas et al. 2019, p. 17). The latest frontier, multilayer networks that accommodate multiple types of interaction and spatiotemporal heterogeneity, multiplies the challenges of obtaining adequate data (Pilosof et al. 2017).

Food-web data are limited in two respects. One is quantity. Despite the rapid growth of network ecology over the last 20 years, the number of empirical data sets in use remains surprisingly small and overwhelmingly wet. Cirtwill et al. (2015) found 196 usable food webs to investigate latitudinal variation in niche width; of these, only 31 (16%) were terrestrial, and those were derived from just 19 primary sources with a mean age of >50 years. The other limitation is data quality—the fatal flaw of first-generation food webs. Many studies now explicitly acknowledge the importance of consistent taxonomic resolution, and contemporary data do contain many more nodes, links, and metadata than did early data (Ings et al. 2009). But it does not necessarily follow that "food-web structure is well described" (Thompson et al. 2012, p. 695). The use of aggregated trophic species persists (de Visser et al. 2011, Jacquet et al. 2016) despite considerable evidence that species sharing exactly the same foods and predators should be virtually nonexistent in nature (see Section 3). Moreover, first-generation food webs are still used for meta-analysis despite their known defects. Critical evaluations of recent data have highlighted inadequacies and asymmetries of sampling effort, which bias inferences by missing rare species and/or interactions (Blüthgen 2010, Fründ et al. 2016, Jordano 2016). This is indeed a crucial issue, but not the only one. Another is whether data gatherers have correctly identified nodes and links (Poisot et al. 2016). There has been little scrutiny of this latter issue, but there is cause for concern.

3. THE ROOTS OF IGNORANCE ABOUT FOOD-WEB STRUCTURE

> Detailed information about the diet of the majority of free-ranging mammals and birds does not exist and often only the most generalized approximation of food items consumed is known.
>
> —Jordan (2005, p. 108)

Jordan's statement is as true today as it was 15 years ago—and it pertains to mammals and birds, which are better known than anything else. A food web is a collection of diets; if detailed dietary information does not exist, then no well-resolved food web is possible. Our ignorance of what wild animals actually eat has multiple causes, some of which have been discussed for decades. Others have gone mostly unmentioned, even if they are widely perceived by field biologists. Still others have only recently become apparent and reveal that our ignorance is even deeper than anyone had realized. In this section, we discuss these causes, focusing mostly on three issues that we believe are underappreciated—at least in their scale and scope. In the process, we touch upon developments in molecular ecology that have brought problems to light but also offer solutions; we expand on these developments in Section 4.

3.1. Taxonomic Impediments and Pseudotaxonomy

> I am also frustrated by working for a half century in the field, nurtured and guided at long distance by the world's best taxonomists, among hundreds of thousands of species of organisms, most of which are actually known to science yet can be identified in the field, at best, by only a select few.
>
> —Janzen (2004, p. 732)

It is exacting work to accurately identify most wild organisms to the species level, even for a taxonomic specialist with a mint-condition specimen. Thus, a major question for food webs is not just how finely resolved the nodes are, but also how and by whom the nodes were identified. Most ecological studies provide little to no methodological information about taxonomic identification, and only a tiny minority involve professional taxonomists or deposit reference specimens in permanent collections (Bortolus 2008). Many community-level studies group look-alikes as morphospecies, which frequently confounds ecologically important distinctions in ways that cannot be reconstructed after the fact (**Figure 1**; Section 3.2).

The frequency and severity of taxonomic misidentifications in the community-ecology literature has never been quantified but is undoubtedly high, even in intensively studied communities (Egli et al. 2020). Gotelli's (2004) personal account of struggling to identify North American ant species resonates with our own experiences. For example, when a list of Kenyan savanna plants compiled by ecologists (Goheen et al. 2013) was refined by DNA barcoding in collaboration with botanists (Gill et al. 2019), a wide variety of identification errors came to light and the list grew considerably (Pringle 2020). This example is probably fairly typical of community-level studies by taxonomic amateurs. In several well-documented cases, such errors have led to flawed biological inferences and catastrophic management decisions (Bortolus 2008). And taxonomic errors propagate once they are etched into the literature and incorporated into public databases (Vilgalys 2003; but see Leray et al. 2019). Knowlton & Jackson (1994, p. 8) pointed out that "'pseudotaxonomy' is just as much an impediment to understanding as 'pseudoreplication,'" but ecology has yet to confront the implications of this impediment.

For most food-web inferences, the correct application of currently accepted Latin names (if those even exist) is not overwhelmingly important. What is essential is that taxa have been

correctly distinguished—or, if taxa are unavoidably lumped, that the boundaries of the lumps are at least knowledgeably defined (Roslin et al. 2013). These things cannot be assumed even for the small set of putatively highly resolved food webs that are commonly used and reused in network analyses.

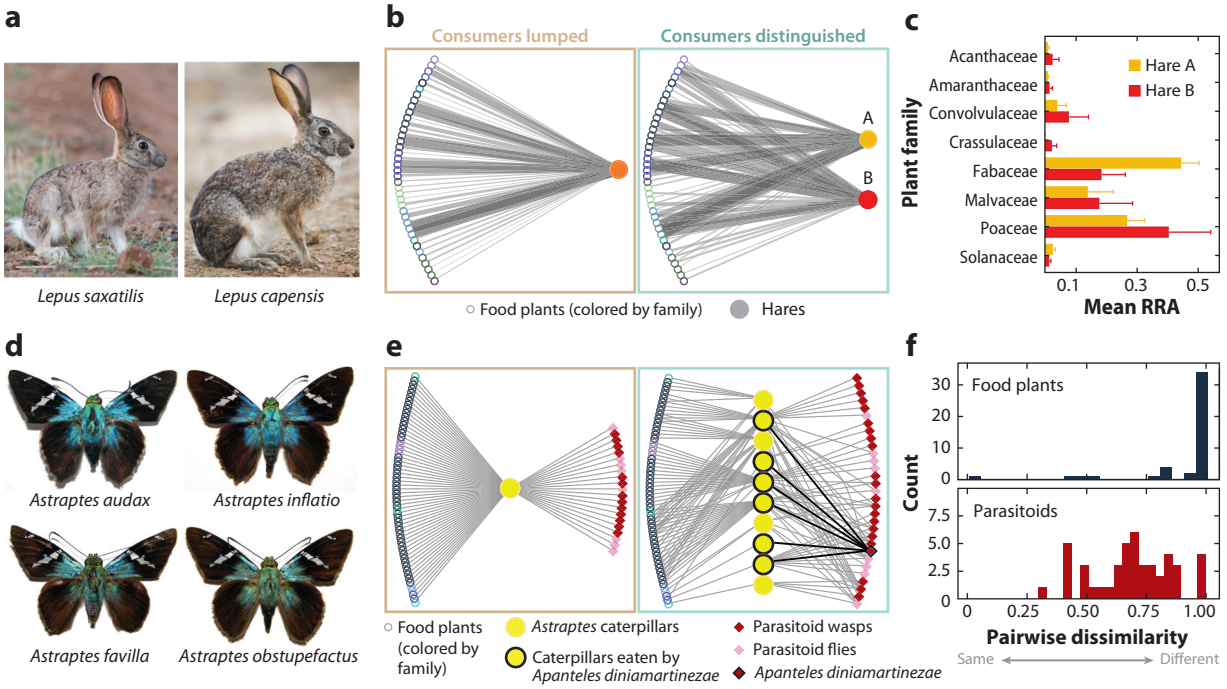

Figure 1

Failure to distinguish cryptic species distorts food-web structure and masks ecological specialization. (*Top row*) Splitting hares alters inferences about diet breadth and composition. (*a*) Scrub hare (*Lepus saxatilis*) and Cape hare (*Lepus capensis*) co-occur in central Kenya but are virtually indistinguishable in the field. Kartzinel et al. (2019) sequenced hare DNA (D-loop of the mitochondrial control region) and plant DNA (P6 loop of the chloroplast *trn*L intron) from fecal samples, which revealed two hare haplotypes with significantly different diets; these types could not be conclusively matched with Latin binomials based on GenBank accessions and were provisionally labeled A and B. (*b*) Bipartite networks show the trophic interactions inferred when hares A and B are lumped (*left*) or distinguished (*right*); food-plant taxa are colored by family, and link widths reflect the relative read abundance (RRA) of plant DNA sequences in fecal samples (data from Kartzinel et al. 2019, including only sequences that accounted for ≥1% of RRA per sample). (*c*) Bar graph shows the mean RRA (+1 SE) of DNA from eight top plant families in samples from hares A and B; the former's diet is dominated by legumes (Fabaceae), whereas the latter eats more grass (Poaceae). (*Bottom row*) DNA barcoding (mitochondrial CO1) resolved 10 morphologically cryptic species of skipper butterfly—lumped for more than 100 years under the name *Astraptes fulgerator*—all from within just 1,260 km^2 in Área de Conservación Guanacaste, Costa Rica (Hebert et al. 2004). (*d*) Adult males of four of these species: *Astraptes audax* (voucher: 02-SRNP-29798); *Astraptes inflatio* (voucher: 02-SRNP-20353); *Astraptes favilla* (voucher: 06-SRNP-55033); and *Astraptes obstupefactus* (voucher: 97-SRNP-1804). Barcoding similarly resolved many cryptic parasitoid species. (*e*) These discoveries transformed the tripartite network of food plants (*left* in each network; colored by family), caterpillars (*center*; *yellow*), and parasitoids (*right*; *red*, wasps; *pink*, flies). Braconid wasps formerly known as *Apanteles leucostigmus* resolved into 36 highly specialized cryptic species (Smith et al. 2008, Fernandez-Triana et al. 2014), one of which, *Apanteles diniamartinezae*, eats six of the cryptic *Astraptes* species (dark outlines in the network on the *right*). (*f*) Histograms show distributions of pairwise Bray-Curtis dissimilarities between caterpillar species in terms of their food plants and parasitoids (1 indicates total difference), showing that the 10 cryptic butterfly species occupy extremely different ecological niches. Photographs in panel *a* reproduced with permission from BIOSPHOTO/Alamy Stock Photo (*left*), Nature Photographers Ltd./Alamy Stock Photo (*right*). Photographs in panel *d* reproduced with permission from D. Janzen and W. Hallwachs.

3.2. The Problem of Cryptic Species

> It makes no sense to describe the interaction structure of nodes which in themselves are poorly defined.
>
> —Roslin et al. (2013, p. 2)

Many currently accepted Latin binomials are in fact complexes of two or more genetically and ecologically distinct lineages that are difficult or impossible to distinguish morphologically (Knowlton 1993). The discovery of such cryptic species has accelerated with the advent of modern molecular diagnostics, especially DNA barcoding (Hebert et al. 2004; Smith et al. 2006, 2007, 2008; Janzen et al. 2017). Cryptic species are not confined to diverse and poorly studied biotas, nor to small and inconspicuous taxa. Indeed, they appear to be remarkably evenly distributed across taxa and biogeographic regions (Pfenninger & Schwenk 2007).

The ubiquity of cryptic species massively compounds the problems associated with inexpert taxonomy and the resulting mischaracterization of biodiversity, co-occurrence patterns, niche relationships, specialization, individual variation, population dynamics, species interactions, and network architecture. When cryptic species are distinguished, seemingly widespread generalist species resolve into multiple more-specialized ones (Knowlton & Jackson 1994, Janzen et al. 2009, Smith et al. 2011). In central Kenya, two outwardly indistinguishable species of hare (*Lepus* spp.) were detected using mitochondrial DNA and were found to have significantly different diets (Kartzinel et al. 2019). Field observations alone would indicate a single hare morphospecies with a broader and more indiscriminate dietary niche than either of the cryptic species actually has (**Figure 1*a–c***). An unresolved complex of cryptic specialists will also appear to be both more numerous and more intraspecifically variable than its constituent species really are. The relationships between abundance, generalization, and network structure is one area of current interest (Fort et al. 2016, Dormann et al. 2017) where a failure to distinguish cryptic species will thwart progress. The causes and consequences of individual variation (Araujo et al. 2011, Clegg et al. 2018) is another.

The implications of cryptic species for inferences about food webs and the biology of the species involved are powerfully illustrated by the plant-caterpillar-parasitoid food web of the dry, rain, and cloud forests of Área de Conservación Guanacaste (ACG) in Costa Rica (**Figure 1*d–f***). An inventory that began in 1978 has reared ~850,000 caterpillars of ~7,000 Lepidoptera species, along with ~3,000 species of fly and wasp parasitoids of those caterpillars (Janzen et al. 2009, Janzen & Hallwachs 2016). For the comparatively well-studied butterflies and moths, 10–20% of morphologically characterized species turn out to be multispecies complexes in light of DNA barcodes and collateral ecological data on habitat affiliation and larval food plants (Hebert et al. 2004; Janzen et al. 2009, 2017). Although there are many variations in the hundreds of such complexes discovered thus far (reviewed in Janzen et al. 2009, Janzen & Hallwachs 2016), the adults are typically morphologically very similar to completely indistinguishable, often including the genitalia and other characters used by taxonomists to describe and identify species, whereas the caterpillars are often morphologically distinct and eat different food plants (sometimes sympatrically and sometimes not). Sometimes morphological features previously assumed to reflect individual variation are discovered to be diagnostic of species differences in the hindsight of genetic information (and sometimes not). The parasitoids of these caterpillars include many more species than previously thought, are much more specialized than previously assumed, are only occasionally generalists, and are mostly undescribed (Smith et al. 2006, 2007, 2008; Fernandez-Triana et al. 2014; Arias-Penna et al. 2019). One of the few (<5%) braconid wasp parasitoids that actually had a name (*Apanteles leucostigmus*) was previously thought to be a generalist consumer of 32 caterpillar species; it resolved into 36 species, each of which eats only one or a few closely related

caterpillar species (Smith et al. 2008). Crypticity can result from shallow divergence, selection for morphological similarity [i.e., mimicry (Janzen et al. 2009)], or simply the lack of selection for any morphological dissimilarity that a human can perceive (Janzen et al. 2017).

Host-parasitoid webs are a staple of the network-ecology literature, in part because they are believed to be empirically tractable and "usually resolved to the level of biological species" (Ings et al. 2009, p. 256). In ACG, this only became true after specimens were routinely DNA barcoded starting in 2004. The 25-year-long, professional-taxonomist-assisted inventory up to that point had been conflating hundreds of cryptic species as well as incorrectly splitting some species in which extreme sexual dimorphism had led taxonomists to assign different names to males and females (Janzen et al. 2009). The lessons of ACG are not peculiar to the tropics (Kaartinen et al. 2010, Smith et al. 2011) or to insects. Killer whales go by one name (*Orcinus orca*) but include multiple genetically differentiated, sympatric types that specialize on different prey (LeDuc et al. 2008). Other examples span everything in between wasps and whales (Pfenninger & Schwenk 2007).

3.3. The Problem of Cryptic Interactions

> Even if one were to stand beside the animal, it would be difficult to identify which individual plants were being eaten.
>
> —Talbot & Talbot (1962, p. 131)

Even for noncryptic species, trophic interactions are difficult to quantify by any means. Visual observations are especially problematic. Organisms that are nocturnal, small, rare, shy, remote, or hidden (in soil, sediments, or other organisms) are all hard to observe. Many interactions happen in the blink of an eye—zap, a lizard ate an insect, but which insect? Others are easy to overlook. Scavengers eat dead meat but also invertebrates within the carrion (Polis 1991). Gut contents and fecal macroremains often can be identified only to family or order (if that). Many interactions are infrequent and atypical and are thus unlikely to be detected or deduced: Leopards eat fish (Balme et al. 2019), sharks eat birds (Drymon et al. 2019), wolves eat grasshoppers (Barton et al. 2020), hippos eat elephants and other hippos (Dudley et al. 2016). Such rare interactions may strongly influence consumer fitness and community dynamics (e.g., Dudley et al. 2016), as well as assumptions about forbidden links in food webs (Jordano 2016). Indeed, weak links and their configuration are thought to be important in stabilizing food webs (McCann et al. 1998, Jacquet et al. 2016; but see Allesina & Tang 2012), and their omission can bias network metrics and inferences about specialization.

Powerful insights about food webs have emerged from studies of animals that eat slowly in plain sight, such as sea otters (Tinker et al. 2008), starfish (Paine 1966), and big cats (Balme et al. 2019). Even for this least-cryptic category of consumers, the issues of Sections 3.1 and 3.2 apply. Moreover, the amount of time and effort required to approach a comprehensive dietary database is far greater than is typical of ecological field studies (**Figure 2**).

Connectance: the proportion of all possible interactions in a network that actually occur

Cryptic interactions can profoundly affect inferences about food-web topology. In an Arctic host-parasitoid web, the addition of molecular diagnostics to a database of rearing records tripled the number of links, the connectance, the number of host taxa per parasitoid taxon (generality), and the number of parasitoid taxa per host taxon (vulnerability) (Wirta et al. 2014). Nestedness increased ninefold. Parasitoids that attack host eggs or pupae were missed by rearing but were detected by sequencing host DNA from adult parasitoids. In other host-parasitoid webs worldwide, DNA barcoding has simultaneously resolved both cryptic taxa and cryptic interactions, with variable effects on overall network architecture (Kaartinen et al. 2010, Hrcek et al. 2011,

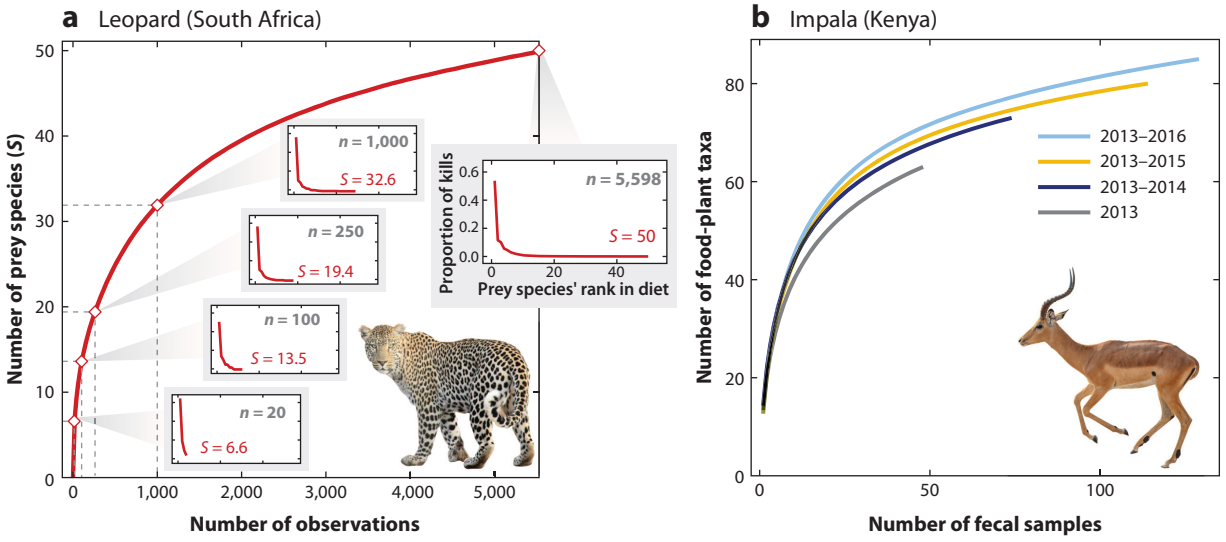

Figure 2

Intensive sampling is required to characterize diet composition and detect infrequent interactions. (*a*) Rarefaction curve of leopard (*Panthera pardus*) dietary richness as a function of sampling effort (n = 5,598 observations of 146 leopards over 6 years) in Sabi Sands Game Reserve, South Africa (data from Balme et al. 2019). Impala (*Aepyceros melampus*) accounted for 54% of kills, and three prey species (all antelopes) collectively accounted for 75% of kills, meaning that leopards contribute a few strong and many weak links to the food web. Many infrequent prey (e.g., birds, catfish, pangolin, hyena, other leopards) were detected only with extraordinary sampling effort. Insets are rank-abundance distributions of diet composition at different sampling intensities (n observations in each inset); the prevalence of dominant prey is conserved across sampling depths, but dietary species richness (S in each inset) decreases sharply as sampling effort decreases. A food web constructed on the basis of even 1,000 observed kills would contain fewer than two-thirds of the prey species actually eaten by this leopard population, thereby omitting many weak links. (*b*) Sample-based rarefaction of dietary richness for impala in Laikipia, Kenya, based on DNA metabarcoding of fecal samples collected over four field seasons [data from Kartzinel et al. 2019, including only sequences that accounted for ≥1% of relative read abundance (RRA) per sample]. Curves show the richness of food-plant taxa after one year (2013, 48 samples, 63 foods), two years (2013–2014, 74 samples, 73 foods), three years (2013–2015, 114 samples, 80 foods), and four years (2013–2016, 129 samples, 85 foods). Metabarcoding enables the identification of many foods with few samples, yet dietary richness nonetheless increases as cumulative sample size grows and infrequent interactions are detected. Photographs reproduced from (*a*) **https://commons.wikimedia.org/wiki/File:Panthera_pardus_(passant_regardant).jpg**; copyright Martyn Seddon (CC0 1.0) and (*b*) **https://commons.wikimedia.org/wiki/File:Trotting_impala_ram.jpg**; copyright 2012 Hein Waschefort (CC BY-SA 3.0 US).

Smith et al. 2011). As in Costa Rica's ACG, the trend is often toward greater specialization, but not always (Wirta et al. 2014). The difference might depend on the prevalence of cryptic species, which by definition cannot be more generalized than the original morphospecies.

Trophic interactions involving large generalist consumers are often cryptic in a different way. It is easy to watch an elephant eating but hard to quantify the diet of an elephant population (**Figure 3**). Early studies of African ungulates, noting that "the observational method proved of very limited value" (Talbot & Talbot 1962, p. 131), analyzed stomach contents of shot animals. In one study of 71 shot elephants in Uganda, the two most abundant foods were "mature grass" and "young grass" (Buss 1961, p. 134)—a family-level taxonomic identification (Poaceae). Other such studies resolved many grasses to genus or species but lumped other food taxa to family or coarser (Talbot & Talbot 1962). Conventional alternatives to shooting large numbers of animals, such as visual observation followed by identification of bitten stems (Kleynhans et al. 2011) or fecal microhistology (Hansen et al. 1985), are notoriously effort-intensive and tricky to get right. The few attempts to build food webs for African savannas have combined records from early studies

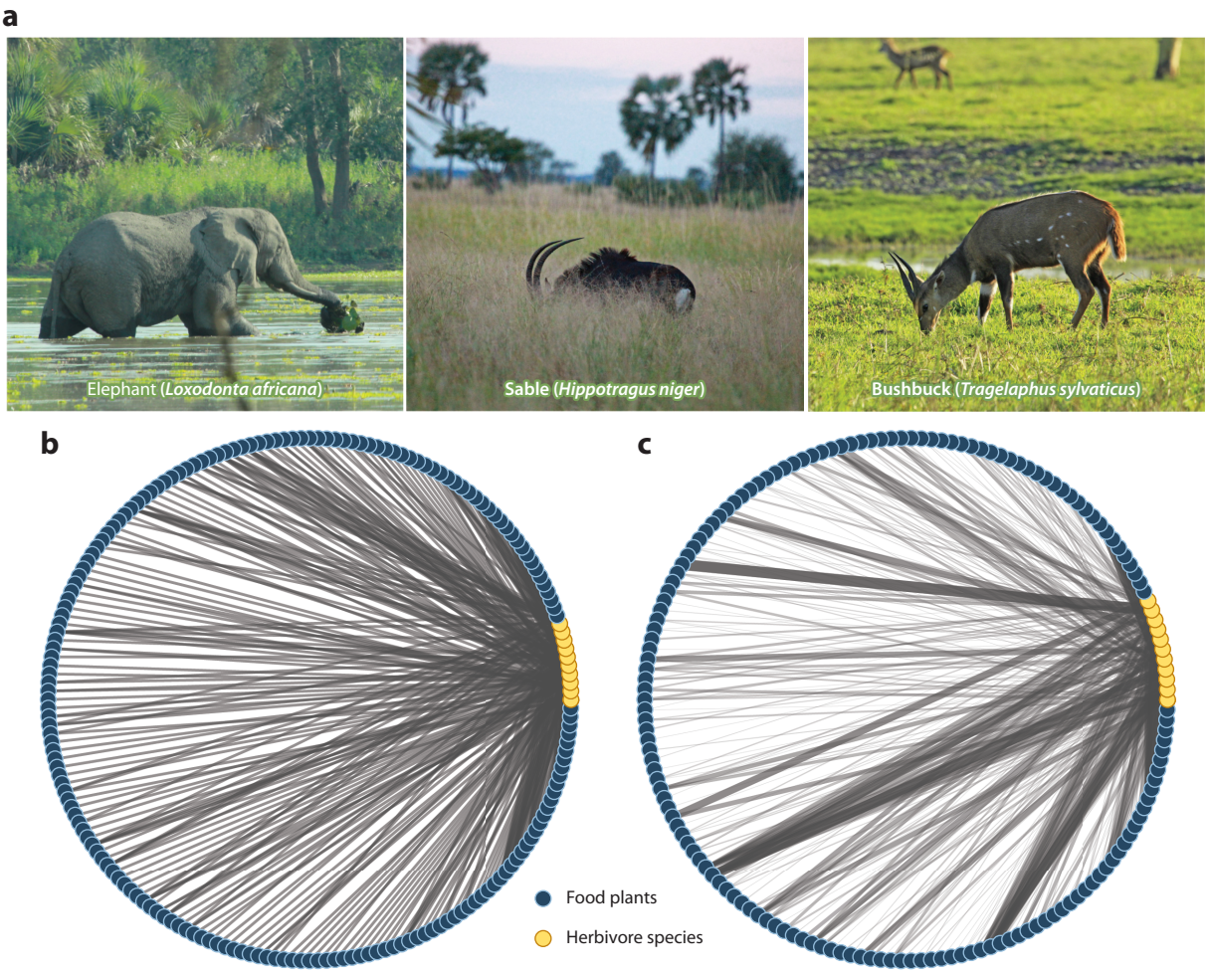

Figure 3

Observational diet assessment is difficult even for the world's largest and most conspicuous land animals. (*a*) Left to right, foraging African savanna elephant (*Loxodonta africana*), sable antelope (*Hippotragus niger*), and bushbuck (*Tragelaphus sylvaticus*) in Gorongosa National Park, Mozambique. Even through powerful binoculars, it is impossible to identify (much less quantify) all plant species being consumed. (*b*) Bipartite network of unweighted (presence-absence) links from observational records of 11 herbivore species (*yellow*) and 181 food plants (*blue*) in Gorongosa over five years (data from Tinley 1977). (*c*) Fecal DNA metabarcoding of the same 11 Gorongosa herbivore species during just one dry season detected 25% more total interactions involving a comparable number of plant taxa ($n = 151$) and enabled the weighting of links using the relative read abundance (RRA) of plant DNA sequences (data from Pansu et al. 2019, including only sequences that accounted for ≥1% of RRA per sample).

to build unweighted networks (Baskerville et al. 2011) or aggregated all basal resources into a handful of categories (de Visser et al. 2011). Recently, fecal DNA metabarcoding has been used to construct highly resolved networks for entire communities of large herbivores, which has clarified the taxonomic and phylogenetic dimensions of diet composition (Kartzinel et al. 2015, 2019; Pansu et al. 2019; Kartzinel & Pringle 2020). Analysis of one such data set reaffirms previous conclusions (Hall & Raffaelli 1993, Martinez 1993, Thompson & Townsend 2000, Winemiller 2007) that the taxonomic resolution of food-web data matters for ecological inference (**Figure 4**).

Cryptic interactions are also common in mutualistic networks. Plant-pollinator networks are overwhelmingly constructed on the basis of observational flower-visitation data. Leaving aside the considerable challenge of correctly identifying and distinguishing all visitors (**Figure 1*d***), such plant-centered sampling can bias the network structure (Jordano 2016). When visitation data have been supplemented with microscopic identification of pollen grains from captured pollinators, network metrics changed markedly (Bosch et al. 2009, Olesen et al. 2011). A DNA-metabarcoding study of insect pollen loads in French heathland quadrupled the number of interactions and altered essentially all metrics of network structure relative to a visitation data set (Pornon et al. 2017). Environmental DNA (eDNA) from flowers can also be used to detect insect visitation

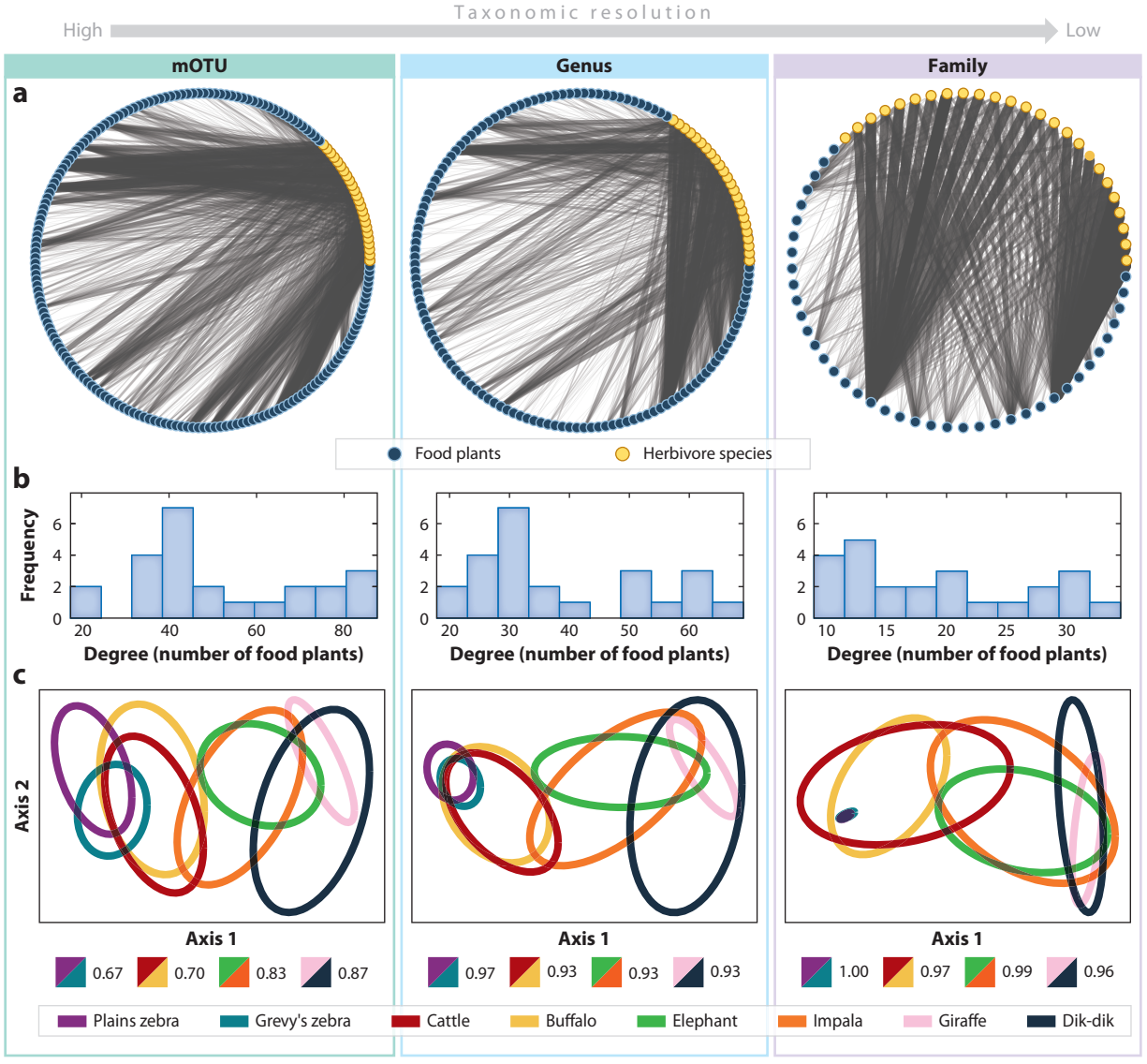

Figure 4

(*Continued*)

(*Caption appears on following page*)

Figure 4 (*Figure appears on preceding page*)

Taxonomic resolution affects food-web structure. Fecal DNA metabarcoding data on plant taxa in large-herbivore diets in Kenya were progressively coarsened from molecular operational taxonomic units (mOTUs, *left*) to genus (*center*) and family (*right*) [data from Kartzinel et al. 2019, including only sequences that accounted for ≥1% of relative read abundance (RRA) per sample]. In this data set, 62% of plant mOTUs matched a single Latin binomial, 27% matched one genus, and 11% matched multiple genera or were resolved only to family. (*a*) Bipartite networks showing links (weighted by RRA) between 24 herbivore species (*yellow*, including elephant and all ungulates represented by ≥10 fecal samples) and food-plant taxa (*blue*; $n = 165$ mOTUs, 121 genera, 41 families). Mean dietary diversity decreases, and network connectance increases, as resolution is coarsened. (*b*) Degree distributions (histograms with 10 bins each, showing the number of herbivore species with a given number of trophic links) shift as taxonomic resolution decreases. (*c*) Nonmetric multidimensional scaling ordinations illustrating the dietary dissimilarity (reflected by separation in the plots) among eight dominant herbivore species (colored 90% confidence ellipses; data points, corresponding to individual fecal samples, are not shown). At mOTU-level resolution, all species pairs exhibit some degree of dietary resource partitioning, which decreases when data are coarsened to genus level; at family-level resolution, the assemblage collapses into three guilds (grazers, browsers, and mixed-feeders) with near-total niche overlap within guilds. Beneath each panel are pairwise Pianka niche-overlap values (1 indicates total overlap) for the four pairs of species (colors) with the most similar diets (plains and Grevy's zebra, Cape buffalo and cattle, impala and elephant, dik-dik and giraffe). (*d*) Network modularity (rows, plant taxa; columns, herbivore species; shading, RRA of each plant taxon in each herbivore diet). Modularity decreases, and the module membership of herbivores (*dashed boxes*) changes, as taxonomic resolution decreases.

(Thomsen & Sigsgaard 2019), suggesting a way to combine individual-level plant- and animal-centered sampling, analogous to the combination of host- and parasitoid-centered DNA barcoding by Wirta et al. (2014).

Non-consumptive predator-prey interactions are important in food webs (Schmitz et al. 2004, Ings et al. 2009, Valdovinos et al. 2010, Pringle et al. 2019). Topologically, these are links that could be strong but are in fact weak or absent owing to anti-predator responses by prey. Such interactions are inherently cryptic, but accounting for them is often essential for understanding ecological dynamics. For example, on small Bahamian islands, an experimental invasion of large predatory lizards decimated populations of two smaller *Anolis* lizard species (Pringle et al. 2019). The intuitive explanation was that the large species simply ate many of the smaller ones. However, PCR assays and DNA metabarcoding of the predator's feces, coupled with isotopic analysis of tail tissue, indicated that the predator mostly ate insects and only rarely ate *Anolis* lizards (which had rapidly moved into arboreal refuges to avoid the predator). This habitat shift intensified competition between the *Anolis* species for space and food, contributing to the decline of both species. In this study, molecular methods were key in documenting both the rarity of intraguild predation and the signatures of interspecific competition (dietary niche shifts and overlaps). This example also illustrates how behavioral responses can complicate efforts to predict network structure on the basis of trait matching or co-occurrence (Pilcher et al. 2020).

3.4. Other Problems

Various other empirical problems degrade the quality of data used for the analysis of food webs and other ecological networks. These have been more frequently discussed, but they have not gone away. Most of them ultimately stem from the enormous effort required to catalog and quantify species interactions in the field.

Sampling intensity is a problem regardless of the methods used to infer links (Blüthgen 2010, Fründ et al. 2016, Jordano 2016) (**Figure 2**). A key question is how to interpret the absence of an otherwise plausible interaction: Is it because that link is forbidden by phenological mismatches or other trait incompatibilities, or is it because the network was studied for too little time with too few observations (Jordano 2016)? Similarly, is a species with only one documented link really an extreme specialist, or were its other interactions simply not observed (Bosch et al. 2009)?

Food webs are spatiotemporally dynamic, but measurements are discrete. A consumer may eat a food in one place or time but not others. Such contingency can be problematic when investigators

pull data from widely dispersed literature sources to assemble food webs [as many do by necessity (Baskerville et al. 2011, de Visser et al. 2011)]. Such amalgamated data are likely to mix and match ecological factors, leading to the assembly of webs that may not have existed in any real place at any actual time.

Data on interaction strengths and on species' traits and relative abundances greatly inform the study of ecological networks, and they are often necessary to resolve ambiguities that arise otherwise (Ings et al. 2009, Dormann et al. 2017, Gaiarsa & Guimarães 2019). But such data are rarely available. Cohen et al. (2009, p. 22335) found only three food webs with "relatively complete trophic link data and with average body mass and population density data for each taxon," and two of them were from the same lake in different years. The number has not increased much, if at all, since then.

Individuals within a population often differ dramatically in diet—depending on size, physiological condition, behavioral plasticity, habitat heterogeneity, species interactions, and other factors—and such intraspecific variation affects food-web structure (Clegg et al. 2018). In populations with pronounced individual specialization, poorly resolved data or insufficient sampling effort may severely distort perceptions of the population-level diet. Within one 0.5-ha field, Roeder & Kaspari (2017) found that fire ants (*Solenopsis invicta*) were extreme trophic generalists, but that individual colonies were consistently specialists ranging from herbivores to predators-of-predators.

3.5. The Upshot and Universality of These Problems

> Food webs are simply proving to be composed of more nodes linked in more ways than we ever knew.
>
> —Roslin & Majaneva (2016, p. 616)

Data on the structure of real food webs have never been good; whether they have been even adequate for the purposes of previous studies is impossible to know until the conclusions of those studies can be tested against data sets that account for the issues described above. Some authors appear to have concluded that the demons of resolution have been vanquished. Thompson et al. (2012, p. 691) implied that the "limitations of older data" had been "dealt with," while Ings et al. (2009, p. 253) celebrated "a new catalogue of evermore complete, taxonomically resolved, and quantitative data." These sentiments are true insofar as the earliest food webs were so crude that any attention to biological detail is an improvement. But research during the last 10 years has exposed how stubborn the demons of resolution really are. Ecologists have not gotten better at taxonomy—if anything, the reverse—and professional taxonomic expertise continues to starve for resources. Cryptic taxa and interactions are ubiquitous, and resolving them can transform network topology and associated inferences about structural generalities, species' roles, niche breadth and overlap, competition and indirect effects, individual variability, generalization and specialization, coevolution, functional redundancy, and many other phenomena that ecologists study (**Figures 1–4**). These issues are common to tropical, temperate, boreal, and polar regions; to terrestrial, freshwater, and marine environments; and to traditional food webs, mutualistic networks, and host-parasitoid networks.

4. OPPORTUNITIES AND CHALLENGES IN FORENSIC FOOD-WEB ANALYSIS

4.1. Molecular Tools for Diet Analysis

Many of the problems reviewed above have been illuminated by the use of molecular methods to resolve network nodes and links, particularly DNA barcoding and metabarcoding, although the

potential arsenal of tools is larger (Pompanon et al. 2012, Roslin & Majaneva 2016, Taberlet et al. 2018). These techniques have been developed and refined by molecular ecologists, but over the last five years they have increasingly merged into the ecological mainstream (Zinger et al. 2019). Here, we provide a minimalist overview of these methods and refer readers to recent reviews for details, caveats, and best-practice guidelines (Cristescu & Hebert 2018, Taberlet et al. 2018, Deagle et al. 2019).

Barcoding and metabarcoding involve the amplification and sequencing of a short and taxonomically diagnostic genomic region (DNA barcode). Barcoding with Sanger sequencing is used for individual organismal samples that yield relatively high-quality DNA and hence longer barcodes [500–1,000 bases for commonly used plant and animal markers (Hebert et al. 2003, Hollingsworth et al. 2011)], which facilitate taxonomic identification. Metabarcoding with high-throughput sequencing is used for complex soups of DNA from many taxa, including bulk organismal samples and eDNA samples such as feces, gut contents, and pollen loads (in which the DNA is often degraded by digestion and/or environmental exposure). Diversity, degradation, and the capacity of high-throughput sequencing platforms necessitate reliance on shorter sequences that can be amplified with conserved primers, and there is generally a trade-off between taxonomic breadth and taxonomic resolution (Pompanon et al. 2012).

Either barcoding or metabarcoding can be used to reconstruct trophic interactions, depending on the study design (e.g., Wirta et al. 2014, Kartzinel et al. 2015). We focus primarily on metabarcoding of fecal and other eDNA samples, which has the greatest potential to transform food-web data because it enables broad-spectrum and high-resolution characterization of vast numbers of samples using standardized methods, with considerable economies of scale (Evans et al. 2016). High-throughput sequence data are typically clustered into molecular operational taxonomic units (mOTUs), which are then identified to the finest taxonomic category possible by comparison to DNA reference libraries. Data can be presented as frequencies of occurrence (the proportion of samples that contain an mOTU) and/or as relative read abundances (RRA; the proportion of sequence reads per sample of an mOTU). These metrics offer complementary information about the intensity of an interaction (Deagle et al. 2019). RRA is often assumed to be a first-order approximation of proportional diet composition. This assumption is strong and loaded with caveats (see Section 4.3); it performs reasonably well in some contexts (Willerslev et al. 2014, Craine et al. 2015, Kartzinel et al. 2015) and worse in others (Deagle et al. 2010). Judicious interpretation is required.

Barcode-based approaches have repeatedly been shown to yield higher taxonomic resolution and accuracy than the available alternatives for characterizing diets (Deagle et al. 2009, Soininen et al. 2009, Newmaster et al. 2013, Wirta et al. 2014). They consistently detect interactions that would otherwise go unrecorded. They enable large sample sizes, which does not bypass the sampling-intensity problem but does alleviate it (**Figure 2**). And they have other desirable features. Fecal samples can generally be collected more easily than animals can be observed (and noninvasively). Fecal samples also provide individual-level data and can thus be used to create individual-based and trait-based networks (Ings et al. 2009). Moreover, frequency of occurrence and RRA provide imperfect but often decent proxies for interaction intensity (Deagle et al. 2019).

Taxonomically verified DNA reference libraries based on vouchered reference specimens are essential to maximize the reliability of DNA-based diet analyses. Global databases such as GenBank (Leray et al. 2019) and BOLD (Ratnasingham & Hebert 2007) are good and getting better. Ideally, each study would create its own local reference library and deposit the specimens in museums and the sequences in public databases, thereby supporting strong system-specific inferences while simultaneously augmenting global repositories. For example, Gill et al. (2019) barcoded 460 of an estimated 500 plant species thought to occur at a Kenyan site and deposited the

1,781 specimens in herbaria, where they were identified by botanists; this local reference database was then used to characterize the diets of the 33-species large-herbivore community via metabarcoding of 1,322 fecal samples (Kartzinel et al. 2019).

4.2. Opportunities

The potential of metabarcoding and allied techniques to inform network ecology is recognized (Evans et al. 2016, Poisot et al. 2016), but the marriage is just beginning. We believe that this union is essential and urgent in light of the problems reviewed in Section 3. With it, we expect to gain not just more complete and taxonomically resolved networks but also fresh insights in a number of conceptual and applied arenas.

4.2.1. Dietary niches: cryptic differentiation is common. Metabarcoding has been used to probe subtleties of niche partitioning and overlap. In Kenyan large herbivores, mOTU-resolution data confirmed a canonical niche axis, proportional grass consumption, but also revealed a distinct axis of niche partitioning at the level of plant species (Kartzinel et al. 2015) (**Figure 4*c***). In Wales, Lucas et al. (2018) discovered resource partitioning between species and genera of generalist hoverflies (Syrphidae), contrary to expectation—and contrary to the common practice of lumping all hoverflies as a single functional group in pollination studies. In Jamaica, insectivorous bats exhibited cryptic dietary separation that corresponded with differences in echolocation behavior (Emrich et al. 2014). Many similar examples are emerging of cases in which the dietary differences between coexisting species were simply never perceivable using conventional methods, which is one reason why the continuing use of aggregated trophic species in network analysis is likely to misrepresent biological reality.

4.2.2. Nutritional ecology. Combining dietary metabarcoding with data on the traits of food items can be used to characterize the nutritional ecology of free-ranging animals, which has long been difficult (Raubenheimer 2011) and is needed for captive husbandry and breeding programs (Jordan 2005). This has recently been attempted for large herbivores in Mozambique's Gorongosa National Park (Branco et al. 2019). Incorporation of data on animals' age, sex, condition, and movements further expands the range of potentially answerable ecological questions (Atkins et al. 2019).

4.2.3. Specialization and individual variation. Because of its sensitivity and because it allows large sample sizes, metabarcoding enhances the detectability of rare interactions that otherwise complicate the diagnosis of population-level specialization, which in turn underpins inferences about coevolution, coextinction, and the causes of network modularity (Dormann et al. 2017). Predictions about populations' robustness or fragility to disturbance may depend on which dimension of dietary specialization is considered. Analysis of one of the most extensive fecal-metabarcoding data sets compiled to date found that large herbivores can be taxonomic generalists (high dietary species richness) but phylogenetic specialists (few dietary lineages) and vice versa (Kartzinel & Pringle 2020).

Metabarcoding data can also be used to evaluate ideas about individual-level specialization, such as the niche-variation hypothesis (Bison et al. 2015, Pansu et al. 2019). However, a single fecal sample or pollen load per individual (integrating some few days of consumption) may often be an insufficient representation of individual niche breadth. The noninvasiveness of fecal sampling creates the opportunity for repeated sampling of known individuals and hence fuller characterization of individual variation. Few studies have yet capitalized on this opportunity.

4.2.4. Mechanistic interpretation of field experiments. Noninvasive sampling is also essential in the context of experimental manipulations that would be disrupted by removing animals from populations. Manipulative experiments are the gold standard for scientific inference, yet the results of ecological field experiments are often mechanistically ambiguous: The addition or removal of a consumer may cause resource species to increase and/or decrease in abundance (Coverdale et al. 2016), but the reasons may be obscure in the absence of information about the consumer's diet. Indeed, the original motivation for using metabarcoding in our own research was to enhance mechanistic insight from such experiments. The Bahamian lizard example discussed in Section 3.3 illustrates how complementary DNA-based dietary information can completely change the interpretation of experimental results (Pringle et al. 2019). In Mozambique, Guyton et al. (2020) used a 5-year metabarcoding data set to reinforce a 3-year herbivore-exclusion experiment, which together showed that native ungulates both heavily consumed and strongly suppressed the invasive shrub *Mimosa pigra*.

4.2.5. Feces as a window. Fecal samples offer a composite glimpse into the life of an animal over the previous few days. The same samples used to evaluate an animal's diet can be used to characterize its genotype, gut microbiome, and parasites, as well as the relationships among these attributes (Srivathsan et al. 2016, Kartzinel et al. 2019). Genotyping can also facilitate longitudinal individual-level diet sampling (it is otherwise possible, but difficult, to be certain that one is sampling the same animal through time). Simultaneous sequence-based assessment of multiple interaction types from the same samples is well-suited for analysis using the kinds of multilayer-network approaches now being developed, with the added advantage of having the data in a common currency (Pilosof et al. 2017).

Fecal metabarcoding can also provide a window into the environment as a form of biomonitoring: The animals sample the environment, and the researcher determines what they found (e.g., Schnell et al. 2012). This application could aid in the early detection of invasive species, among other things.

4.3. Challenges

There are many technical and bioinformatic challenges inherent in the production and curation of high-quality sequence data for diet analysis; these topics require dedicated treatment (e.g., Taberlet et al. 2018), and we do not review them here. Instead, we focus on several obstacles to ecological inference that arise even after sequence data of the highest possible quality have been generated and curated.

4.3.1. Limits to taxonomic resolution. Although metabarcoding and other DNA-based diet-analysis methods provide higher resolution than their alternatives, they are never perfect. The choice of DNA region and PCR primers influences taxonomic resolution, and resolution often varies across taxa in the diet. Barcodes that target a wider range of taxa tend to resolve fewer mOTUs to species level. For example, vertebrate-specific primers may yield species-level resolution in 72% of cases, whereas mammal-specific primers would increase that fraction to 86% (Taberlet et al. 2018). In addition, primers can mismatch food taxa to varying degrees, resulting in differential amplification. Differential digestibility and DNA copy number among food taxa and tissues influence how much of their DNA is recovered, although this issue cuts both ways: Conventional methods of scat analysis may be even less likely to detect highly digestible items (Deagle et al. 2009). Sometimes prey-of-prey are detected in a predator's feces (Bowser et al. 2013), and such secondary predation can influence conclusions about food-web topology.

4.3.2. Validation. As noted above, food-web data need interaction strengths, and RRA is a potentially usable proxy. It is a proxy at best, because true interaction strength describes the effect of one species on another (Novak & Wootton 2010), although the term is often used more loosely. Even to be a generally reliable proxy, the interpretation of RRA must be validated in a wider range of contexts than it has been to date. RRA is affected by primer and amplification biases, differential digestibility, and variable DNA copy number across different food taxa and tissues. A flotilla of controlled-feeding experiments (Deagle et al. 2013, Willerslev et al. 2014) could help to clarify when and to what extent RRA reflects proportional diet composition, and thus when and where the use of experimentally derived correction factors (Thomas et al. 2016) would be most valuable. The same experiments would generate information on gut-retention times, which is valuable for the biological interpretation of metabarcoding data.

4.3.3. Consumption versus selection. Diet composition is most meaningfully interpreted in light of data on the relative availability of different foods. Metabarcoding data have been combined with availability data to estimate consumer selectivity (Pansu et al. 2019), but this is nontrivial. Quantifying availability at scales relevant to many foraging animals requires enormous effort. The availability of different food types can also be surprisingly difficult to estimate in a common currency, and matching dietary sequences with organisms in field surveys is complicated when mOTUs are not unambiguously assigned to a single Latin binomial—as some invariably will not be, even with the aid of a comprehensive local reference library (**Figure 4**).

4.3.4. When taxonomic resolution is not enough. Herbivores do not eat Latin binomials (Janzen 1979)—or anyway, only rarely. Consumption of different tissues of the same food species (root, fruit, shoot, etc.) is an important mechanism of niche partitioning in ungulates and other animals and is a major determinant of nutrient acquisition, but such differences cannot be resolved by current molecular methods. The same is true for cannibalism, which is common in many systems and has long been a thorn in the side of food-web theory. Whether a consumer kills or scavenges its food is often an important distinction but not one that can readily be detected in fecal DNA. All of these issues remain limitations to the use of DNA alone to reconstruct trophic networks and derive inferences about foraging behavior.

4.4. Complementarity of Multiple Approaches to Diet Analysis

Some of the challenges reviewed in the previous section can be ameliorated by supplementing molecular analysis with complementary methods. We have argued that field observations alone are an insufficient basis for building food webs, but observations remain valuable. Many kinds of technology-enhanced observations have proven useful for gathering and interpreting dietary data, including camera traps, GPS collars, and animal-mounted acoustic or video recorders. Stable-isotope analysis is an immensely valuable tool for many purposes in food-web ecology, such as inferring the length of food chains and the coupling of energy channels (Post 2002, Rooney et al. 2006, Pringle & Fox-Dobbs 2008), and isotopic data can reveal aspects of food-web organization that DNA data cannot. Analysis of fatty acids and other biomarkers may likewise be useful in this regard (Traugott et al. 2013, Nielsen et al. 2018). Like metabarcoding (Zinger et al. 2019), these methods have their own limitations, caveats, inferential pitfalls, and best practices that are sometimes overlooked in the rush to deploy powerful technologies in the field (Martínez del Rio et al. 2009). That all methods of diet analysis have blind spots is a strong argument for combining them in mutually reinforcing ways.

In recognition of the shortcomings of existing food-web data sets, researchers have begun trying to predict trophic interactions using machine-learning algorithms and other approaches

(Desjardins-Proulx et al. 2017, Pilcher et al. 2020). These methods are not a substitute for better empirical data on network structure, which at the least are required to train and validate algorithms, but they have potential to complement DNA metabarcoding and compensate for some of the specific deficiencies discussed above (Bohan et al. 2017).

5. CONCLUSIONS

> A decade ago, it really did look as though there were repeated patterns in food webs. . . . Now, with much better empirical data, prompted by the earlier (some would say premature) generalisations, the whole thing looks much more uncertain.
>
> —Lawton (1999, p. 182)

Food-web ecology has oscillated between mania and depression for 50 years. We now understand many things better than we once did, but the limitations of empirical data and the demons of resolution have blocked anything approaching the depth of insight that ecologists have sought to provide. Often, it has seemed as if the data we have been hoping for are finally arriving or just around the bend (Pimm et al. 1991, Ings et al. 2009), whereupon new problems come to light and make the whole enterprise look cartoonish (Lawton 1999). In this review, we have argued that currently available data on food-web topology should be regarded with extreme suspicion—as a generally unreliable and at best uncertain basis for empirical inference about most of the questions that ecologists have been asking. New problems have come to light, and many old problems are as bad as they were before. Cryptic species are everywhere—even career taxonomic specialists frequently cannot tell them apart—and often these species eat totally different foods, which means that lumping them distorts food-web structure (**Figure 1*d–f***). Cryptic interactions are everywhere, and once they are detected, network topology can change completely (Wirta et al. 2014). The best-documented examples of these problems come from the study of host-parasitoid networks, which have been studied intensively in part because (ironically) they have been considered exemplary of the best possible data, in which all nodes are resolved to species and all links are quantifiable (Ings et al. 2009). None of the few hundred food webs compiled for recent analyses of network structure or in widely used repositories [e.g., Web of Life (**http://www.web-of-life.es**), Interaction Web Database (**https://www.nceas.ucsb.edu/interactionweb/**), or GlobalWeb (**https://globalwebdb.com**)] can be assumed to be entirely free of these same problems.

At the same time, it really does seem to us that more reliable data are finally trickling in and that a bigger wave is about to break. Our perception of the problems with existing data has grown precisely because molecular ecologists have begun delivering better data. The ability to sequence large amounts of eDNA at low cost is a radical break from the conventional methods of field ecology, just as the ability to sequence genomes is a break from the methods used for generations in phylogenetics and systematics. There has never been any comparable advance in the ability to detect and catalog previously invisible trophic interactions; it is like the invention of the microscope. The word revolutionizing (Zinger et al. 2019) is apt. We are aware that utopian predictions seem quaint in retrospect, and a cursory review of the limitations of DNA metabarcoding (Section 4.3) shows that it is not a miracle pill; there are limits to the resolving power of any method for characterizing feeding relationships in nature and limits to the level of resolution that is conceptually sensible to consider. Nonetheless, we believe that it will at least soon be possible to know the extent to which the conclusions of recent studies are sensitive or robust to the most serious deficiencies in the existing body of food-web data.

We have a few concluding thoughts. Molecular diagnostics are necessary but not sufficient for characterizing community and food-web structure. Field studies should strive to DNA barcode vouchered reference specimens of the focal species, which will increase rigor and produce

unexpected insights. Field ecologists studying the resource use of herbivores, carnivores, pollinators, and seed dispersers should DNA metabarcode feces and pollen loads, at least until even better methods emerge. This will rapidly lead to the accumulation of more and better network data. Network analysts should be highly discriminating in the data sets that they use, read the methods of the primary sources, and judge whether the procedures are likely to have adequately resolved nodes and links. There is a need for judgment about what is an adequate level of empirical resolution to rigorously test a particular hypothesis. Which metrics and inferences are most sensitive to the most common kinds of empirical errors? How robust are conclusions to a plausible range of error in node identification and link structure? In general, we would expect to learn more biology from three good food webs (Cohen et al. 2009) than from hundreds of globally distributed webs of highly uncertain and uneven quality and completeness.

In this last regard, we advocate the development of a few genuine model systems—in the sense of *Escherichia coli*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Mus musculus*—for food-web ecology. These would be a handful of ecosystems where multiple research groups work independently and collaboratively on generating and analyzing the complete eukaryotic food web using the full range of molecular, nonmolecular, experimental, and nonexperimental tools available. Tuesday Lake and Ythan Estuary are obvious candidates (Cohen et al. 2009). Serengeti, or at least some slice of it, might be another (Dobson 2009). Small Caribbean islands also have appealing properties (Pringle et al. 2019). If there really are universal structural properties and organizing principles of food webs, then a model-systems approach should help to reveal their mechanistic basis. It has worked in genetics.

That structure affects function is one of the most fundamental truths in science (Strogatz 2001). Network ecology is the right idea (Bascompte & Jordano 2014, Poisot et al. 2016) but has always been too far ahead of the data needed to support it. We and others (Evans et al. 2016) perceive a new reason for optimism. The rapid but until-now independent advances in molecular ecology and network theory are merging. Harnessing these methods to the conceptual wheels that have long been turning in community ecology may enable a great leap forward in our understanding of complex ecosystems.

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Volume 51, 2020

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Stefan A. Talke and David A. Jay

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Malin L. Pinsky, Rebecca L. Selden, and Zoë J. Kitchel

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Sinéad Collins, Philip W. Boyd, and Martina A. Doblin

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The Emerging Ecological and Biogeochemical Importance of Sponges on Coral Reefs
Joseph R. Pawlik and Steven E. McMurray

The North Atlantic Ecosystem, from Plankton to Whales
Andrew J. Pershing and Karen Stamieszkin

The Nutritional Ecology of Marine Apex Predators
Gabriel E. Machovsky-Capuska and David Raubenheimer

Improving Predictions of Salt Marsh Evolution Through Better Integration of Data and Models
Patricia L. Wiberg, Sergio Fagherazzi, and Matthew L. Kirwan

Vascular Plants Are Globally Significant Contributors to Marine Carbon Fluxes and Sinks
Simon M. Cragg, Daniel A. Friess, Lucy G. Gillis, Stacey M. Trevathan-Tackett, Oliver M. Terrett, Joy E.M. Watts, Daniel L. Distel, and Paul Dupree

The Biological Pump During the Last Glacial Maximum
Eric D. Galbraith and Luke C. Skinner

From the ***Annual Review of Microbiology***, Volume 74 (2020)

Ape Origins of Human Malaria
Paul M. Sharp, Lindsey J. Plenderleith, and Beatrice H. Hahn

Archaeal DNA Replication
Mark D. Greci and Stephen D. Bell

The Plant Microbiome: From Ecology to Reductionism and Beyond
Connor R. Fitzpatrick, Isai Salas-González, Jonathan M. Conway, Omri M. Finkel, Sarah Gilbert, Dor Russ, Paulo José Pereira Lima Teixeira, and Jeffery L. Dangl

What Is Metagenomics Teaching Us, and What Is Missed?
Felicia N. New and Ilana L. Brito

The Influence of Bacteria on Animal Metamorphosis
Giselle S. Cavalcanti, Amanda T. Alker, Nathalie Delherbe, Kyle E. Malter, and Nicholas J. Shikuma

Toward a Fully Resolved Fungal Tree of Life
Timothy Y. James, Jason E. Stajich, Chris Todd Hittinger, and Antonis Rokas

Prospects and Pitfalls: Next-Generation Tools to Control Mosquito-Transmitted Disease
E.P. Caragata, S. Dong, Y. Dong, M.L. Simões, C.V. Tikhe, and G. Dimopoulos

A Bacterial Tower of Babel: Quorum-Sensing Signaling Diversity and Its Evolution
Nitzan Aframian and Avigdor Eldar

Conflict, Competition, and Cooperation Regulate Social Interactions in Filamentous Fungi
A. Pedro Gonçalves, Jens Heller, Adriana M. Rico-Ramírez, Asen Daskalov, Gabriel Rosenfield, and N. Louise Glass

The Ingenuity of Bacterial Genomes
Paul C. Kirchberger, Marian L. Schmidt, and Howard Ochman

From the ***Annual Review of Phytopathology***, Volume 57 (2019)

Revisiting the Concept of Host Range of Plant Pathogens
Cindy E. Morris and Benoît Moury

Genome Editing, Gene Drives, and Synthetic Biology: Will They Contribute to Disease-Resistant Crops, and Who Will Benefit?
Kevin V. Pixley, Jose B. Falck-Zepeda, Ken E. Giller, Leland L. Glenna, Fred Gould, Carol A. Mallory-Smith, David M. Stelly, and C. Neal Stewart Jr.

Durability of Quantitative Resistance in Crops: Greater Than We Know?
Christina Cowger and James K.M. Brown

Molecular Dialog Between Parasitic Plants and Their Hosts
Christopher R. Clarke, Michael P. Timko, John I. Yoder, Michael J. Axtell, and James H. Westwood

Ecology and Evolution of the Sudden Oak Death Pathogen *Phytophthora ramorum*
Niklaus J. Grünwald, Jared M. LeBoldus, and Richard C. Hamelin

The Evolution, Ecology, and Mechanisms of Infection by Gram-Positive, Plant-Associated Bacteria
Sbree P. Thapa, Edward W. Davis III, Qingyang Lyu, Alexandra J. Weisberg, Danielle M. Stevens, Christopher R. Clarke, Gitta Coaker, and Jeff H. Chang

Understanding Adaptation, Coevolution, Host Specialization, and Mating System in Castrating Anther-Smut Fungi by Combining Population and Comparative Genomics
Fanny E. Hartmann, Ricardo C. Rodríguez de la Vega, Fantin Carpentier, Pierre Gladieux, Amandine Cornille, Michael E. Hood, and Tatiana Giraud

Interactions and Coadaptation in Plant Metaorganisms
M. Amine Hassani, Ezgi Özkurt, Heike Seybold, Tal Dagan, and Eva H. Stukenbrock

Surviving in a Hostile World: Plant Strategies to Resist Pests and Diseases
Samuel W. Wilkinson, Melissa H. Magerøy, Ana López Sánchez, Lisa M. Smith, Leonardo Furci, T.E. Anne Cotton, Paal Krokene, and Jurriaan Ton

From the ***Annual Review of Plant Biology***, Volume 71 (2020)

Regulation and Evolution of C_4 Photosynthesis
Urte Schlüter and Andreas P.M. Weber

Modeling Plant Metabolism: From Network Reconstruction to Mechanistic Models
Teresa J. Clark, Longyun Guo, John Morgan, and Jorg Schwender

Evolution of Plant Hormone Response Pathways
Miguel A. Blázquez, David C. Nelson, and Dolf Weijers

Salt Tolerance Mechanisms of Plants
Eva van Zelm, Yanxia Zhang, and Christa Testerink

Molecular Mechanisms of Pollination Biology
Róisín Fattorini and Beverley J. Glover

Sequencing and Analyzing the Transcriptomes of a Thousand Species Across the Tree of Life for Green Plants
Gane Ka-Shu Wong, Douglas E. Soltis, Jim Leebens-Mack, Norman J. Wickett, Michael S. Barker, Yves Van de Peer, Sean W. Graham, and Michael Melkonian