Trends in Parasitology

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Opinion

A dearth of data: fitting parasitoids into ecological networks

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Studying parasitoids can provide insights into global diversity estimates, climate change impacts, and agroecosystem service provision. However, this potential remains largely untapped due to a lack of data on how parasitoids interact with other organisms. Ecological networks are a useful tool for studying and exploiting the impacts of parasitoids, but their construction is hindered by the magnitude of undescribed parasitoid species, a sparse knowledge of host ranges, and an under-representation of parasitoids within DNA-barcode databases (we estimate <5% have a barcode). Here, we advocate the use of DNA metabarcoding to construct the host–parasitoid component of multilayer networks. While the incorporation of parasitoids into network-based analyses has far ranging applications, we focus on its potential for assessing ecosystem service provision within agroecosystems.

Highlights

Parasitoids are key ecosystem service providers within sustainable agriculture and integrated pest-management strategies due to their function as biocontrol agents.

There is a dearth of data regarding how parasitoids fit within wider communities of interacting species, but such information is essential for the successful implementation of conservation biological control in open-field agroecosystems.

DNA barcoding is a useful tool for the establishment of host–parasitoid interactions (and many other associations) and can enable the rapid and relatively cost-effective construction of ecological networks.

Ecological networks, specifically multilayer ecological networks constructed using DNA-based methods, can significantly aid our understanding of how land management can influence multiple ecosystem services and lead to enhanced agricultural sustainability.

Utility of host–parasitoid networks with a focus on agriculture

Host–parasitoid dynamics have been a major focus of ecological and evolutionary study since the early 20th century due to the essential role parasitoids play within ecological communities, and their function as biocontrol agents (Box 1). The economic value of natural pest control is estimated to be $4.5 billion annually in the USA alone [1]. With recent bans on insect pesticides, as well as the prevalence of insecticide resistance, usage of biocontrol agents is likely to increase in the near future [2]. Release of parasitoids to control pests in closed systems, such as the widespread use of *Encarsia formosa* to control greenhouse whitefly (*Trialeurodes vaporariorum*) [3], is standard agricultural practice, and a range of parasitoid species are commercially available. Similarly, parasitoids have successfully been used in **classical biological control** (see Glossary). *Anagyrus lopezi* has been spectacularly successful against cassava mealybug in Africa, with savings estimated between US$8 billion and US$20 billion over 40 years [4]. Parasitoids are increasingly being discussed in the context of **conservation biological control** (**CBC**) [2,3,5]. However, the focus of host–parasitoid research mostly concerns direct interactions between agricultural pests and their parasitoids (Figure 1A). Relatively little is known about interactions between parasitoids and non-pest hosts (i.e., complete host ranges), other parasitoids, and predators (Figure 1B,C) [6] occurring within agricultural and natural systems. There is a growing realisation that these 'non-target' interactions can influence the utility of parasitoids as natural biological control agents via indirect effects, that is, interactions acting between two species that are mediated by one or more additional species (Figure 2) [7–9]. The impacts of these indirect interactions have been shown in experimental and field settings; for example, Sanders and van Veen (2012) found the absence of one parasitoid species can lead to the extinction of another via competitive exclusion between their two host species [9], and Cronin (2007) demonstrated that two hosts can impact one another's population via shared parasitoids [8], an indirect effect called 'apparent competition' which has long been hypothesised to impact the dynamics of pest populations [10].

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Box 1. What are parasitoids?

Insect parasitoids comprise a large number of species and are defined by their larval feeding strategy – that is, they feed exclusively on an arthropod host, almost exclusively leading to its death [23]. They are mostly found within the orders Hymenoptera, Diptera, and Strepsiptera, but of these, Hymenopteran parasitoids are the best studied. Parasitoids are of vital importance for biocontrol [68], there is mounting recognition of their role in pollination services [69], and they have been successfully used as bioindicators for the wider health of ecosystems [18].

Insect parasitoids display a variety of life-history strategies leading to obscure parasitoid complexes. Askew and Shaw (1986) [70] grouped parasitoids into two types based upon whether the host is killed or paralysed during oviposition (idiobiosis) or whether the host can continue to develop after oviposition (koinobiosis). Idiobiont parasitoids are often (though not always) ectoparasitic, that is, their larvae do not develop within the host but are external to it. These species are generally believed to display a greater host range than koinobiont parasitoids, which are typically endoparasitic [23]. Further, parasitoids can display primary parasitism, in which a single parasitoid directly attacks a single host; superparasitism, in which multiple individuals of the same parasitoid species attack the same host individual; multiparasitism, in which multiple parasitoid species attack the same host individual; or hyperparasitism, in which one parasitoid will attack the larva of another parasitoid within a nonparasitoid host, as well as variations around each of these life histories [71]. Due to this complexity, the study of parasitoid interactions is fraught with difficulties, but molecular approaches have the potential to overcome many of these.

The rise in development and application of **ecological networks** (Box 2) over the past two decades is indicative of their utility for analysing complex questions in ecology and evolution [11]. Their application to host–parasitoid systems has provided valuable insights into impacts of anthropogenic drivers of ecosystem change beyond agricultural systems, such as habitat modification within tropical forests [12] and the effect of climate change within arctic communities [13]. But the use of networks to link species, habitats, and ecosystem services within an agricultural context could facilitate decision-making at both the landscape and local scales. The construction of ecological networks that reflect the specificity and frequency of interactions within natural systems enables the quantification of direct and indirect effects on population trends, attack rates, and **ecosystem services** like pollination and biological control [7,11,12,14], and could provide a paradigm shift in the holistic management of **agroecosystems**. For example, knowing the complete host range of a parasitoid species can inform how its population can be bolstered by the presence of non-pest hosts [7] or predict how it might impact native communities through non-target effects if introduced as a biocontrol agent [15], and understanding which parasitoids share host species enables us to study competition and how this reduces overall pest control function [16]. The study of hosts and parasitoids with no known impacts upon agricultural systems could not only benefit ecosystem services within cropland via unknown indirect effects, but can also help to uncover the impacts of climate [13] and land use change [17,18] upon ecological communities in the context of conservation.

A deeper understanding of parasitoid interactions at the community level is therefore required before CBC can be fully incorporated into viable **integrated pest management** (**IPM**). This is of particular importance in light of contemporary policy directives promoting the use of CBC in sustainable agricultural practices globally [5,19].

DNA barcoding (Box 3) can reduce the time and cost of constructing host–parasitoid networks by aiding the identification of parasitoid species and enabling the establishment of trophic links between parasitoid and host [20]. It can also help to overcome the bias of traditional rearing approaches and enable the characterisation of difficult-to-observe interactions [21]. With the development of new high-throughput sequencing approaches, plus the increased availability and reduced cost of these tools as the field progresses, their utility is continuing to grow [22]. Yet, the benefit of molecular taxonomy for host–parasitoid research is significantly lessened by a poor representation of parasitoid species within global barcode repositories relative to global parasitoid diversity.

Glossary

Agroecosystem: an ecosystem within agricultural land.
Classical biological control: the introduction of exotic biocontrol agents into a new region to control an invasive pest.
Conservation biological control (CBC): the application of land management practices that encourage populations of pest natural enemies (predators, parasitoids, and pathogens) to thrive.
Cryptic species: species which cannot be distinguished from one another using traditional morphological approaches and are often only revealed as distinct upon examination of molecular data.
DNA barcoding: the method of using PCR to amplify short fragments of DNA in order to identify and compare species.
DNA metabarcoding: similar to DNA barcoding but involves the amplification of DNA fragments from multiple species simultaneously using next-generation or high-throughput sequencing.
Ecological network: representation of interacting species where nodes typically depict species, and links typically depict a single type of biotic interaction between them.
Ecosystem service multifunctionality: the simultaneous provision of multiple ecosystem services in relation to their demand by humans.
Ecosystem services: the positive benefits conferred by ecological systems to humans.
Integrated pest management (IPM): strategies that incorporate biological, traditional, and chemical practices to control pests to minimise environmental damage.
Multilayer network: ecological network comprising multiple types of nodes or links; for example, they can represent interacting species across spatiotemporal gradients where each spatial or temporal class is represented by a layer, and inter-layer links are distinct from within-layer links.
Multiplex network: a type of multilayer network comprising multiple types of species interaction such as pollination, predation, parasitism, and herbivory.
Reference barcode: a DNA barcode sequence from a specimen accurately identified to species level using traditional morphological taxonomy.

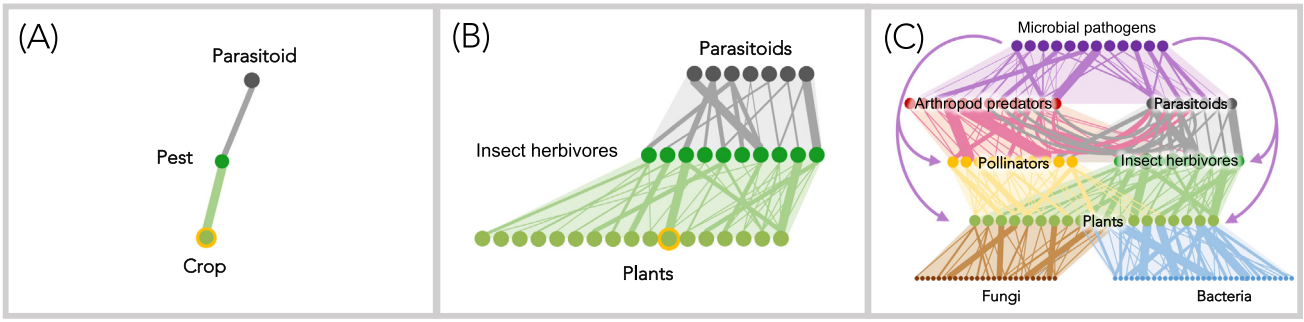

Figure 1. Fitting host–parasitoid interactions into multilayer ecological networks. Illustration of how research focus has shifted from (A) a simple crop plant–pest–parasitoid interaction in isolation, to (B) a network approach showing how a crop plant (green node encircled by yellow) is indirectly connected to other plant species by shared insect herbivores and how these herbivores are indirectly connected to one another via shared parasitoids. A further extension of this approach is to use (C) multilayer networks to simultaneously consider multiple interaction types by including symbiotic microbes, pollinators, other predators and microbial pathogens.

In this Opinion paper, we advocate for the increased use of DNA barcoding to construct host–parasitoid networks in ecological studies. Better representation of parasitoid species in public DNA repositories will facilitate their incorporation into ecological networks and their study across ecosystems, including agricultural systems for the management of ecosystem services. We discuss the key obstacles in the study and application of host–parasitoid interactions and the factors that have led to the current situation, before making suggestions for enhancing our knowledge base using molecular methods, specifically DNA barcoding and **DNA metabarcoding** approaches. By combining advances in molecular and network ecology, we show how this information could be included within advanced 'multilayer' ecological networks that incorporate multiple interaction types, habitats, and human decision-making for a range of sustainable agricultural applications.

The key obstacles in parasitoid–host ecology

Key challenges to the study of host–parasitoid networks, both within agricultural systems and beyond, are (i) the prevalence of undescribed parasitoid species, largely due to the difficulty of species-level morphological identification; (ii) lack of host–parasitoid association data for most parasitoid species; (iii) the comparatively low proportion of parasitoid species with a DNA barcode; and (iv) the effort involved in establishing links using traditional approaches.

Insect parasitoids comprise a huge number of species, mostly found within the insect orders Hymenoptera, Diptera, and Strepsiptera, and are defined by their larval feeding strategy – that is, they feed exclusively on an arthropod host, leading to its death [23]. Morphological taxonomic expertise in arthropods is in relatively short supply [24], and this is especially true for Hymenoptera [25], the order to which most parasitoids belong. The study of hymenopteran taxonomy is often recognised as having been neglected when compared to the other three large insect orders. A 'difficulty of engagement', their typically small size, and a lack of taxonomic literature, coupled with their uncharismatic nature, seems to have deflected many would-be hymenopterists away from the challenge [26]. Our knowledge is skewed towards the Aculeata, comprising bees, ants, and eusocial wasps, and away from the remaining Apocrita which contains the megadiversity of mostly parasitoids [27]. Additionally, seemingly high levels of **cryptic speciation** exhibited by parasitoids suggest that actual levels of diversity are higher than currently predicted [28]. In 1991, LaSalle and Gauld [26] produced a particularly compelling argument calling for the incorporation of parasitoid Hymenoptera into future research strategies. They ended their plea with a number of recommendations including more research into basic taxonomy and the formation of a database listing species inventories. Almost 20 years later, this group is still severely understudied.

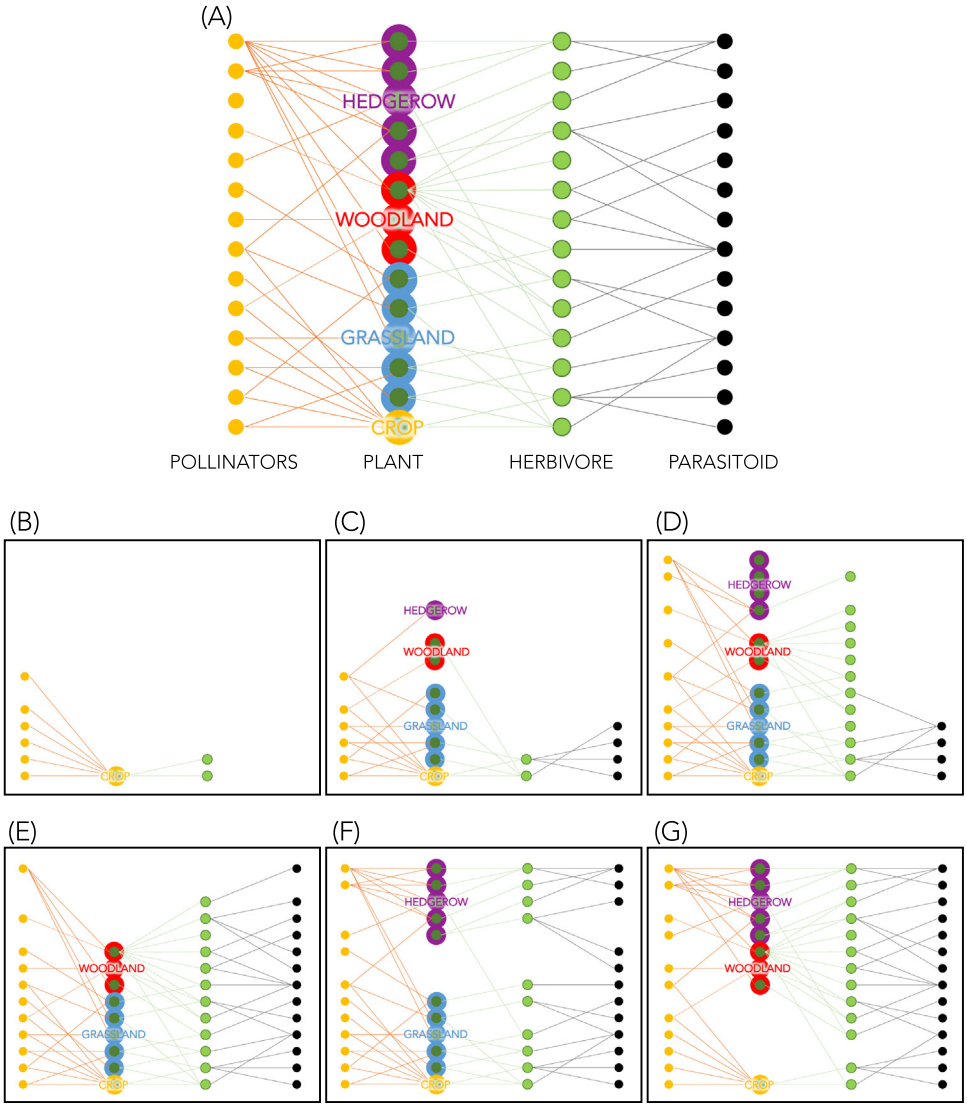

Figure 2. The importance of a whole-ecosystem approach: indirect effects and multiple habitat types. (A) Example of a multilayer network combining data from plant–pollinator, plant–herbivore, and herbivore–parasitoid networks within four farmland landscape habitat types: woodland, hedgerow, grassland, and crops. (B–D) The progression of species and interactions considered for primary (direct) interactions with a crop plant species (B), secondary (indirect) interactions with a crop plant species (C), and tertiary (indirect) interactions with the same crop plant species (D). (E–G) The effect of removing non-crop habitats on direct and indirect interactions: hedgerow (E), woodland (F), and grassland (G). Considering whole communities across multiple habitat types reveals that crop plants display many 'hidden' interactions.

The deprived state of parasitoid taxonomy has clear implications for specimen identification within individual ecological sampling projects [29]. Traditionally, specimen identification requires advanced understanding of parasitoid morphological taxonomy: a restricted skill set and a time-consuming venture. But, with the advent of molecular methods and high-throughput sequencing, the adoption of a combined approach utilising both DNA barcoding (Box 3) and traditional morphological taxonomy is becoming increasingly popular. This method involves generating cytochrome oxidase 1 (COI) barcode sequences for specimens collected in the field and relating these to a reference database of sequences generated from morphologically

Box 2. Ecological networks

Ecological networks characterise the interactions that occur between coexisting species such as predation, herbivory, pollination, and parasitism (Figure I). They deepen our understanding of community structure by revealing the strength and nature of the connections between organisms. This information can enhance our ability to anticipate and mitigate the effects of both anthropogenic and natural environmental change on biological communities.

Ecological networks, in this context, comprise nodes (species) connected by links (interactions). These links can simply represent the presence of an interaction (qualitative networks) or the relative strength of an interaction (quantitative networks). Link strength in host–parasitoid networks is typically established using the frequency of association. That is, the proportion of individual larvae that have been parasitized by a given parasitoid species. Networks comprising different interaction types are traditionally studied independently, but there is a growing realisation that this approach limits predictive ability. Because the ecological and evolutionary dynamics of a community are dependent upon all interaction types present at a given time, combining multiple 'subnetworks' of, for example, plant–pollinator or host–parasitoid into a single 'multiplex' network can reveal greater insights into the relationship between species interactions and community composition, with a growing toolbox for analyses drawn from advances in complexity science.

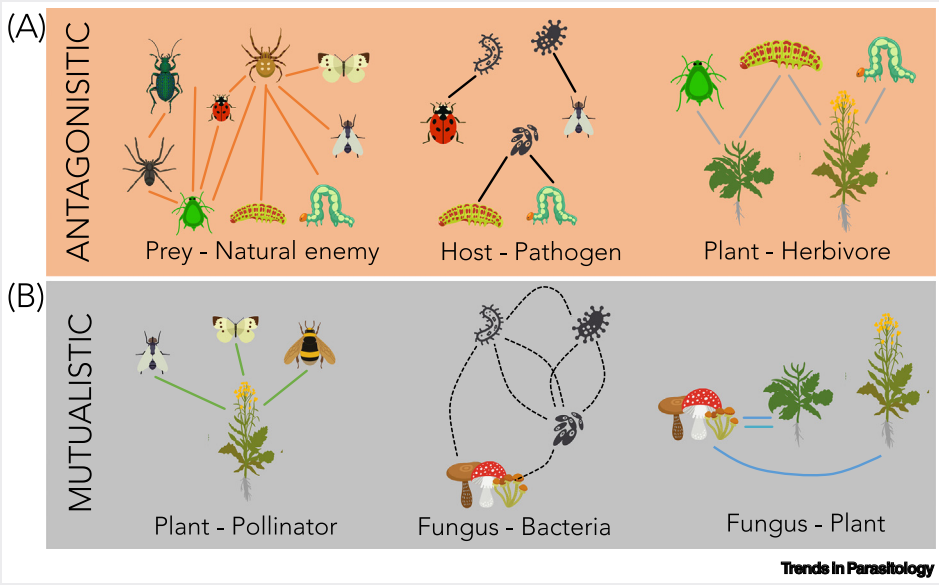

Figure I. Examples of (A) antagonistic ecological networks, and (B) mutualistic ecological networks.

identified specimens [20] such as GenBank [30] or the Barcode of Life Data System (BOLD) [31]. The advantage of this method is a reduced dependence upon taxonomic experts as well as reduced subjectivity and the ability to detect cryptic speciation. While promising, the approach is reliant upon the existence of a reference database comprising species from the study in question and is therefore hindered by the fact that DNA barcodes exist for only a relatively small proportion of parasitoid species. The BOLD database currently holds the barcodes of 319 113 species spanning animals, plants, fungi, and protists. Using this database, we estimate the percentage of hymenopteran parasitoids with barcodes to be between 1.3% and 4.4% of the total predicted number of extant species. These estimates are based upon current hymenopteran parasitoid species diversity figures [23,25,26,32] and the number of species with publicly available COI barcodes in the BOLD database (see Box S1 and Table S1 in the supplemental information online). In addition, there is geographic bias to these accessions, with most specimens coming from North America, mainly from a single study [29]. The result of this situation is that when new parasitoid specimens are collected their DNA barcodes often cannot be matched to existing barcodes within the reference database. Consequently, it is not possible to assign relevant

Box 3. DNA (meta)barcoding

DNA barcoding, that is, the use of a genetic 'barcode' to identify an unknown species, is a popular molecular tool used across the globe [72]. In metazoa, this barcode is usually a 658 bp length of the COI gene dubbed 'the Folmer region' [73]. Taxonomic classification of sequences involves their comparison with a reference database containing sequences of the same locus derived from morphologically identified specimens. The most commonly used of these databases is Barcode of Life (BOLD) [31]. In order to confidently describe insect communities and enable comparability between sampling efforts in different parts of the globe, international reference databases such as BOLD need to be well populated with morphologically described species.

Metabarcoding is a common biomonitoring tool used for determining species composition in environmental samples [74]. It is the parallel amplification and subsequent parallel sequencing of barcodes from multiple organisms simultaneously in order to rapidly characterise the approximate species richness and composition of a mixed sample. Unlike barcoding, which classically utilises Sanger sequencing to generate individual barcode sequences (though this is changing [75]), metabarcoding requires high-throughput sequencing platforms such as Illumina®, Pacific Biosciences®, or Oxford Nanopore Technologies®.

While the standard COI barcoding region is 658 bp in length, the maximum sequence that can be generated by, for example, Illumina sequencing, is realistically 550 bp when taking account of read overlap and quality control. Since the standard COI barcode region is therefore too long to be fully sequenced, many studies utilise mini-barcodes that typically vary between 130 and 500 bp in length [76]. It should be noted that their reduced length means that mini-barcodes do not provide as much genetic information, which can impact their ability to resolve species differences [77].

Once sequences are generated, similar sequences are typically clustered into OTUs, or filtered to create amplicon sequence variants (ASVs) [78], before being linked to sequences within a reference database that have an assigned taxonomic identity [79]. A variety of algorithms exist for the purpose of reducing noise, and revealing which species are present, and their efficacy varies depending upon the composition of species assemblages. For example, some clustering algorithms rely on the percentage of sequence similarity with a relatively arbitrary cut off, usually leading to inaccuracies in the assemblage descriptions. However, (meta)barcoding remains a useful tool for the rapid identification of species and, in some cases, is the only option available.

species-level information to specimens, such as known hosts, habitat preferences, or other life-history traits. Further, species cannot be linked between studies, impeding the detection of large-scale trends, and OTUs (operational taxonomic units) cannot be checked to ensure that they represent 'real' species (Box 3).

Macroecological host–parasitoid studies often aim to reconstruct host–parasitoid networks under divergent conditions, for example within different agricultural crops [12], under controlled warming [33], or along elevational or latitudinal gradients [34]. Until recently, the only method available for the establishment of links between hosts and their parasitoids was direct observation, usually via the rearing of larval hosts. This approach can be extremely effective, and has been used to reveal many novel insights, but is time-consuming and requires detailed knowledge of host species requirements, especially if live specimens are collected at early developmental stages and need to be kept alive until pupation. The approach therefore necessitates a limited taxonomic or functional focus to well-studied host species. Hosts that are more challenging to collect, or rear, are inevitably neglected. Additionally, rearing only establishes a link between the host and the parasitoid that emerges from it, but this can be deceiving. Many parasitoids die before emergence, and secondary parasitoids or hyperparasitoids can emerge from an herbivorous host, masking information about primary parasitoids. DNA barcoding has been used to overcome some of these pitfalls [21,35] and this is our next focus.

Generating sharable, comparable, host–parasitoid data

Molecular approaches to establishing species identity

Traditional DNA barcoding is used to identify individual specimens and, to date, barcode sequences have typically been generated using Sanger sequencing. The limitation of this approach is that each specimen must be prepared separately (DNA extracted, PCR amplified,

cleaned, and sequenced) which, while necessary when constructing a **reference barcode** for a morphologically identified specimen, can be prohibitively expensive and time-consuming when simply identifying specimens by comparing their barcode sequence with those in the reference database. As outlined in Box 3, metabarcoding is the simultaneous sequencing of many specimens using a high-throughput sequencing platform. Due to the greater number of sequences that can be produced simultaneously, this approach is cheaper than traditional barcoding when considered 'per specimen' and enables the rapid identification of many specimens which can be tagged with unique sequence combinations to retain ecologically relevant information such as specimen identity or location [20].

While OTUs, which are loosely correlated with 'real' species [36], need not be taxonomically identified to be useful (for example, network structural properties can be compared between locations by simply using OTUs as nodes), the ability to link them to biological species unlocks associated ecological information, providing greater insights into the study system [37]. However, given the current state of parasitoid systematics, plus the efforts required to fully populate the BOLD COI database with reference barcodes for all extant parasitoid species (keeping in mind that most parasitoid species have not yet been described), it seems prudent to find an alternative or temporary solution that could overcome some of the immediate obstacles for host–parasitoid network analysis. One such suggestion is to use species proxies, essentially global OTUs, based on COI sequences, that are not always linked to a morphologically described species but incorporate sequences generated from multiple studies, globally. Newly generated sequences could be compared to these, enabling comparisons to be made among disconnected studies. BOLD contains such a system called the BIN database [38] which uses a series of algorithms to produce OTUs that very closely relate to true species. While this approach varies in its efficacy for different taxonomic groups, largely due to variability in interspecific sequence divergence [39], it has been successfully used to delineate and identify cryptic speciation in a range of arthropod groups [40–42] and has already proven to be a valuable tool when assessing parasitoid communities [43].

Molecular approaches to determining species interactions

In addition to molecular taxonomy, barcoding and metabarcoding can also be used to establish the links between parasitoids and hosts in a number of different ways (Figure 3). Firstly, barcoding can be used to identify parasitoids that have been reared from hosts, or the hosts themselves, when morphological identification is difficult or cryptic speciation is expected [7,12,43]. This approach has been used within an agricultural context to evaluate the importance of non-crop habitats for pest control [44] and cross-habitat indirect effects [7]. The molecular techniques involved are identical to those described previously for identifying individual parasitoid specimens and are similarly limited by rearing methods.

Conversely, metabarcoding can be used to detect parasitism and multiparasitism before parasitoids emerge and can therefore be used to detect parasitoid DNA from host specimens collected directly from the field without any need for rearing. According to this approach, larvae, pupae, mummies (the remains of parasitised aphids left behind after parasitoid emergence), or the remains of gallers/leafrollers [45] are collected and sequenced individually using universal or parasitoid-specific primers so that the resulting sequences will include DNA from any attacking parasitoid. The method has been successfully applied to aphid, lepidopteran, and hemipteran hosts, helping to reveal how climate can impact farmland host–parasitoid networks [46], and enabling earlier detection and more accurate estimates of attack rates in crop pests [47]. This information could potentially be used to predict pest population growth and assess the necessity of insecticide spraying within IPM strategies in a similar way to how the naturally occurring entomopathogen, *Neozygites fresenii* can regulate cotton aphid populations in the southeastern USA [48]. An important advantage of this

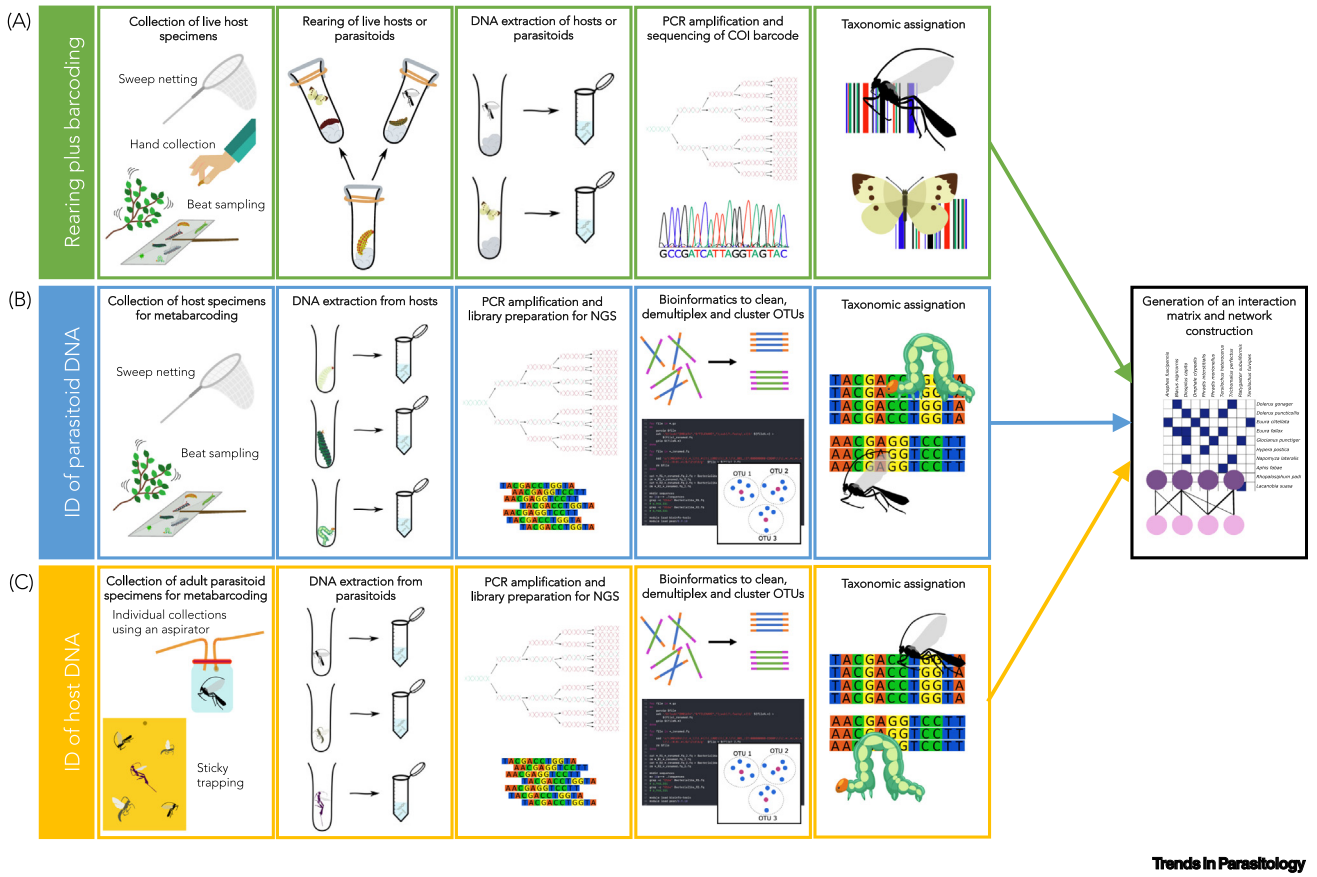

Figure 3. Barcoding and metabarcoding approaches for the construction of host–parasitoid networks. (A) Barcoding can be used to identify parasitoid specimens after they have been reared from host larvae or pupae, or to host remains postparasitoid emergence. (B) Metabarcoding can be used to obtain parasitoid DNA from larvae without the need for rearing or (C) from adult parasitoids caught directly in the field. Abbreviations: NGS, next-generation sequencing; OTU, operational taxonomic unit.

approach is the detection of multiple parasitoid species within the same host, that is, primary or secondary parasitoid and hyperparasitoids simultaneously [47,49], enabling the study of parasitoid complexes (Box 1).

Metabarcoding can also be used to detect host DNA from adult parasitoids, although so far, studies typically use traditional barcoding with host-specific primers [21]. While some refinement of this method is required to avoid contamination from non-host DNA (leading to 'false-positive' interactions) and minimise parasitoid DNA in the resulting sequences [50], the approach has the potential to significantly expand the construction of host–parasitoid networks by providing a more parasitoid-centred view of these interactions. Indeed, previous studies have found that utilising multiple sources of information can provide a less-biased and more complete network [21].

An additional advantage of molecular approaches is the ability to create phylogenetically structured networks, permitting the consideration of coevolutionary dynamics within an ecological community [51] (though see Box S2 for a discussion of barcoding versus whole-genome sequencing). This information can provide insights into the mechanisms that structure communities, such as exploitative competition or habitat filtering [52], and can therefore aid the prediction of environmental change impacts. For instance, phylogenetically structured aphid–parasitoid networks have been used to

test the phylogenetic conservatism hypothesis (that closely related natural enemies attack closely related prey) [53].

Taken together, these approaches enable the study of a greater diversity of host species and can uncover the full host range of parasitoid species. Although we focus here upon the utility of parasitoids for biocontrol in an agricultural context, parasitoids are key drivers of arthropod community composition and dynamics across all biomes, and these methods can shed light on their role in ecosystems where arthropod communities are poorly described [13,54].

While the advantages are clear, it is important to be aware of the limitations of any method. Molecular approaches require access to specialist equipment and expertise which may not be available to all. Additionally, the detection of parasitoid DNA within a host does not necessarily mean that a parasitoid would have killed and emerged from it; there is some suggestion that certain parasitoids regularly lay their eggs within non-host species where they fail to develop [55], while competition between parasitoids within a host can make it difficult to predict which species would have finally emerged. It is important to follow good barcoding practices [50] to minimise the risk of finding nonpermissible interactions, or 'false positives', due to contamination. As such, we do not advocate the complete replacement of traditional rearing approaches with molecular techniques, rather that these approaches are complementary, and that rapid assessment with metabarcoding may serve as a useful indicator of where traditional rearing and morphological taxonomy efforts should be targeted [56]. In fact, the continued efforts of traditional taxonomists, particularly in the discovery and naming of species, rather than being devalued by the popularity of molecular approaches, should be considered essential for their successful application.

Multilayer networks, community dynamics, and ecosystem services

Numerous ecosystem services, other than pest control, are underpinned by ecological interactions, for instance pollination [57], and microbial mutualisms can enhance plant productivity [58] while microbial pathogens of insects can indirectly reduce crop damage [59]. The simultaneous consideration of multiple interaction types can provide an improved understanding of the processes driving ecological community composition and dynamics across landscapes. There is empirical evidence to support this assertion; Bastolla *et al*. (2009) showed that integrating competition between pollinators in plant–pollinator networks predicts overall species richness better than only considering the mutualistic interaction between pollinators and plants [60]. Data also suggest that anthropogenic land-use change or management interventions have divergent effects on different interaction networks [61], implying that decisions made to enhance, for example, pollination, may inhibit pest control or vice versa.

Multilayer networks, that is, networks comprising multiple interaction types or entities [62], provide a framework for management decisions taking **ecosystem service multifunctionality** into account via the evaluation of trade-offs between several ecosystem services simultaneously [62,63]. For example, the creation of flower strips alongside agricultural fields to increase natural pollinator densities may simultaneously increase parasitoid densities if some of the flowering plants also serve as food plants for non-pest parasitoid hosts. But this can only be achieved if the full host range of economically important parasitoids is known.

Applying this concept to host–parasitoid networks in agricultural systems is particularly relevant given the multitude of interactions in which parasitoids partake. Clearly, herbivorous host–parasitoid interactions are the best studied interaction type for their relevance for insect pest suppression. But, taking a more holistic view of the system, it is also clear that these interactions could impact

pollination services through a reduction in caterpillar densities and subsequent butterfly and moth populations. Similarly, parasitoids attack predatory insect larvae and can therefore influence predation rates. Many adult parasitoids require nectar to survive and may act as pollinators themselves. This directly links them to flowering plant species and indirectly to other pollinators and herbivores. Recent studies have highlighted that endosymbiotic bacteria can influence host–parasitoid interactions and shape food webs [64,65]. The use of molecular approaches can aid in the determination of multiple interaction types simultaneously. For example, when extracting DNA from herbivore specimens to detect parasitoid DNA, plant DNA from mouthparts or gut contents may also be detected, leading to the establishment of a tripartite plant–herbivore–parasitoid interaction.

Multilayer networks can be of further benefit when used to examine the reciprocal influence of species from distinct but interacting communities by combining data from multiple habitat types. Typically, host–parasitoid research focuses on a single crop, even though crop fields exist within a rich tapestry of habitat types and landscape features. Again, multilayer networks provide a framework for analysing how crop pests and ecosystem service providers utilise resources at a landscape level (Figure 2E–G). For example, robustness analyses can help to identify disproportionately important plants and habitats in farm species-interaction networks, allowing targeted management and restoration [53,66].

Moving from single species pest–parasitoid dynamics studies though to **multiplex networks** that incorporate plant–host, plant–pollinator, host–parasitoid, predator–prey, and even microbial interactions (Figure 1C), as well as multiple habitat types, will not only deepen our understanding of the impacts of environmental change on agroecosystems but will provide new ways of actively improving management practices to enhance resilience and maximise multiple ecosystem service provision [53].

Concluding remarks

With the growing pressures on land use, an understanding of how organisms and ecosystem services are connected has never been more important. Parasitoids are already widely used as biological-control agents in greenhouses as targeted control of particular pests. Recent developments in both molecular and network ecology provide an unprecedented opportunity to better understand and manage the economic benefits provided by parasitoids in open-field agriculture. However, our knowledge of how parasitoids fit within ecosystems in terms of their direct and indirect interactions with other species is lacking (see Outstanding questions).

It is becoming increasingly clear that the next step in the study of parasitoid–host interactions is to incorporate them into multilayer networks combining multiple subnetworks of differing entities and interaction types [62,67] which can be further extended to examine the reciprocal influence of species from different communities by combining data from multiple habitat types within landscapes. This has immediate applications for farm management and restoration. Given the urgent need to rapidly characterise empirical networks across a variety of landscape and habitat types, using DNA metabarcoding to both identify species and establish interactions is a research priority, especially in the context of facilitating reproducibility and the global sharing of data via online platforms.

Outstanding questions

How many species of parasitoid currently exist when taking account of cryptic speciation?

What is the congruence between molecular and morphological species classifications for parasitoids?

What are the host ranges of parasitoid species, when considering all potential hosts rather than economically important or logistically convenient host groups?

What level of intraspecific variability exists in realised parasitoid host ranges, and what are the drivers of these differences?

How pervasive are indirect effects mediated by parasitoids in managed agricultural landscapes, and what is their relative effect on host population densities?

Do indirect effects mediated via parasitoids permeate across habitat boundaries to impact herbivore host (e.g., pest) insect densities?

What other organisms do parasitoids interact with in highly resolved ecological networks?

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Declaration of interests

The authors declare no competing interests.

Supplemental information

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