

# A network perspective on the vectoring of human disease

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## Abstract

Blood-sucking insects are important vectors of diseases with biting Diptera alone causing an estimated 700,000 human deaths each year. Insect vectors also bite non-human hosts, linking them into host-biting networks. While the major vectors of prominent diseases such as malaria, yellow fever, dengue and Zika are intensively studied, there has been limited focus on the wider interactions of biting insects with non-human hosts. Drawing on network analysis and visualisation approaches from food web ecology, we discuss the value of a network perspective for understanding host-insect-disease interactions, with a focus on Diptera (fly) vectors. Potential applications include highlighting pathways of disease transmission, highlighting reservoirs of infection, and identifying emerging and previously unrecognised vectors.

## **The emergence and spread of zoonotic disease**

More than 60% of known human infectious diseases and 75% of emerging infectious diseases are zoonotic, spreading from animals to humans [1,2]. Recent epidemics, including Zika fever, chikungunya and dengue fever, highlight how novel or apparently stable zoonoses can emerge, creating major challenges to human health and economic wellbeing [3]. Blood-feeding arthropods have been implicated in the spread of at least a quarter of all such outbreaks during the last century [4].

Over the coming century, increased human population densities, greater movement of both people and insects, and the expansion of agriculture and habitation into new areas are likely to favour the emergence, spread and success of novel and existing insect-vector zoonoses [5]. Here we argue that a multi-species analysis of vector-host interactions will help us understand disease epidemiology, minimise risk, and inform control strategies.

## **Moving beyond pairwise interactions between species**

Most research on insect-vector zoonoses has focused on the epidemic and **epizootic** (see Glossary) role of known vector species and their pairwise interactions with their hosts, particularly humans [3,6,7]. However, insect vectors are embedded within complex ecological communities [8] and their interactions with a wider set of species are likely to have important consequences for disease transmission. Ecological interaction network modelling [9] is a useful tool for understanding the interdependencies within these sets of interacting species, and for predicting the dynamics of the component species. Networks

provide a visual and mathematical representation of interactions between **nodes** (e.g. species) connected by **edges** (e.g. feeding interactions)[10]. Networks may be unweighted, where edges are either present or absent, or weighted by the strength or frequency of interactions [11,12]. Both mutualistic interactions (such as interactions between plants and their pollinators) and antagonistic interactions (such as those between predators and their prey) can be represented in this way [13,14].

Using biting Diptera, which currently cause approximately 700,000 deaths annually [15], as a case study, we focus on networks of antagonistic interactions between biting Diptera and the host animals from which they obtain blood meals, referred to here as ‘host-biting networks’. Network ecology can reveal how community structure and function change in response to a range of perturbations, with widespread applications in conservation and agriculture [9]. We suggest that increased application of network approaches in disease vector research will provide valuable insights for understanding vector-borne diseases.

## **The global network of biting interactions**

Host species are connected indirectly through their shared parasites [16]: multiple parasite species can share the same host species [17], and individual parasite species can interact with multiple host species [18]. To illustrate how hosts and parasites are interconnected on a global level, we constructed networks of biting Diptera-host interactions using data compiled from published literature (Figure 1). In total, these data comprise 26,049 biting Diptera-host interactions (where an interaction is defined as the blood of a host individual recorded in the blood meal of a biting insect) from 67 publications involving fieldwork in 32 countries. The resulting network has 263 biting Diptera species, 244 host species, and 1,331

links between host species and biting Diptera species. Biting Diptera interact with host species from five classes: Amphibia, Insecta, Aves, Mammalia, and Reptilia. Cattle (*Bos taurus*) are the most commonly documented hosts, accounting for 6,902 (26.6%) of all interactions. Host species are highly interconnected through shared vector species, with an unweighted network **connectance** of 0.02, meaning that 2% of all globally potential host-insect interactions have been observed. In reality, many potential links will not be possible because of geographic or temporal separation of hosts and biting Diptera, so the true connectance value will be higher. The average unweighted **degree centrality** (the number of links a species has in a network) for the 263 insect species is 5 (range: 1 to 86) host species. A high degree centrality indicates that an insect bites a large number of host species.

While the blood meal data summarised in Figure 1 do not document pathogens within blood meals, nor vector competence in transmitting them, we inferred disease vectoring potential from VectorBase, a database of insect vectors of different diseases [19]. Insects in our network are known to transmit 55 diseases, 39 of which are transmissible to humans (top and middle sections of Figure 1). Within our network, 190 insect species are known vectors of these 55 diseases, of which 154 can be transmitted to humans; most of these insect species (106) were indeed recorded as biting humans in the data compilation.

### **How embedded are humans?**

There are clear epidemiological benefits to characterising the degree to which humans are embedded within networks of host-biting interactions. Increasing contact of humans with livestock and wildlife populations is likely to facilitate a rise in the transmission of emerging diseases [20]. Examples of such vector-driven **disease spillover** events include Lyme disease

and West Nile Virus [21]. Unrestricted and repeated interactions between human hosts and **competent vectors** can allow diseases to reach epidemic levels rapidly [22].

Our compilation of blood meal data reveals that humans are highly embedded in networks of blood-feeding, and interact frequently with well-connected vectors for a variety of diseases (Figure 1). After cattle, humans were the second most frequently-documented host species in the global network, accounting for 6,217 (23.9%) of the interactions involving 152 biting Diptera species. Diptera recorded as biting humans had a significantly wider variety of non-human host species (mean: 6, range 0 to 85) than those that did not (mean: 3, range 1 to 23; Mann-Whitney U = 6448.5,  $p < 0.05$ ). The number of insect-human interactions reported is likely to be skewed by the anthropocentric focus of many of the studies from which data were extracted. However, even mosquito species typically considered anthropophilic (e.g. *Anopheles gambiae* and *Aedes aegypti*) [23–27] interact with a range of non-human hosts (9 and 10 respectively) including, birds, reptiles and non-human mammals.

While biting Diptera can show specific feeding preferences [28,29], many species will feed opportunistically, with consequences for disease control measures [28,30]. Indeed, the five biting Diptera species with the most recorded interactions (*Anopheles arabiensis*  $n=2,280$ , 8.8%, *Culex annulirostris*  $n=1,986$ , 7.6%, *Cx. pipiens*  $n=1,698$ , 6.52, *Aedes camptorhynchus*  $n=1,159$ , 4.5%, and *Ae. aegypti*  $n=1039$ , 4%) had a range of non-human hosts (4, 25, 85, 14 and 10, respectively) which comprised 84.5%, 95.5%, 86.5%, 99%, and 14.8% of their interactions, respectively.

Interpolation (rarefaction) and extrapolation (prediction) curves [31,32] indicate how comprehensively the host ranges of these individual species have been documented (Figure

2A). Observed host ranges were underestimated relative to those predicted through extrapolation by 4, 61 and 3 species for *Cx. annulirostris*, *Cx. pipiens* and *Ae. aegypti*, respectively. This suggests that the known range of host use for even the most commonly-sampled species is still not complete, and highlights the extent to which generalist species such as *Cx. pipiens* might interact with previously unsuspected hosts. No such differences between documented and predicted host species richness were found for *An. arabiensis* and *Ae. camptorhynchus*. The large number of undiscovered hosts for *Cx. pipiens* likely reflects their preference for feeding on birds [33], a particularly species-rich vertebrate class.

Rarefaction at the community level indicates that biting Diptera and host species are relatively well-resolved, with species accumulation curves in each case approaching an asymptote (Figure 2B). In contrast, documented interactions between vectors and hosts are much less complete. We therefore have an incomplete understanding of the full range of vector-host interactions, and further sampling will be necessary to reveal these cryptic interactions and to increase the overall resolution of host-biting networks.

The network shown in Figure 1 contains interactions from a global dataset. In reality, geographically-separated hosts are unlikely to co-occur spatially to the extent that a single Diptera individual can interact with them. However, some hosts (e.g. migratory birds) and humans may be highly mobile. Therefore, it is feasible that hosts that never co-occur could be linked indirectly by a single mobile human individual interacting with a variety of hosts and vectors in different locations. Inevitably, human movement, agricultural encroachment, illegal trafficking of hosts via the bushmeat and wildlife trade, and accidental introductions

of invasive species result in increased opportunities for animal-human interactions and facilitate zoonotic disease transmission [34].

### **Using networks to identify emerging vectors and disease**

As human populations expand into new areas, there is increased opportunity for people to interact directly with novel potential disease vectors, and to interact indirectly, via shared biting insects, with other host species [35]. For example, deforestation and road development in the Peruvian Amazon has increased the frequency of human interaction with the malaria vector *Anopheles darlingi* 278 times relative to intact forested habitat, leading to an increase in malaria cases [36]. The integration of novel species into interaction networks is widely studied in community ecology and conservation biology, where it has broad implications for understanding the impact of invasive species. The influence of a new species depends on the degree to which it is a generalist or specialist and might also be conditioned on the diversity of available hosts [28], with generalists expected to have greater impacts on network structure and dynamics [37,38]. Quantitative network data, combined with information on biting behaviour and vector competence, will be useful to identify novel or encroaching vectors, and their likely disease transmission potential.

The emergence of novel diseases is hard to predict [39], but targeted vector surveillance at the interface of humans and wildlife, informed by data on species' biting preferences, has the potential to help predict and limit the emergence and spread of zoonotic disease. For example, following the rapid spread of *Culex* mosquito vectored West Nile Virus in the United States, intensive surveillance of mosquitoes and their hosts allowed outbreaks to be anticipated and effective vector control measures to be implemented [40]. Regular

monitoring for pathogens of people, domestic animals [41] and wildlife [42] could provide an 'early warning system' for disease emergence. Such an approach could be complemented by mining data associated with molecular analysis of blood meals, focusing on likely vector species identified using weighted network data. Key species to identify in this way will include those that are highly connected and which interact frequently with disease-susceptible hosts (e.g. those with high degree centrality)[43]. In the longer term, data from such a surveillance programme could provide valuable general insights into spatial patterns of disease spillover, temporal variations in vector-host contact patterns, and the relative importance of local and long-range transmission events [44].

Interaction networks could also help highlight unexpected or important hosts, as well as situations where vector populations have different host use in different geographic locations [45]. Hosts that are bitten by many biting Diptera species are candidates for the long-term persistence of disease and infection of sympatric **reservoir hosts** [46]. Identifying these species could therefore inform preventative measures, paralleling the situation where domestic dogs are vaccinated to reduce rabies transmission to humans [47]. Just as for novel vectors, the impact of novel hosts could be determined through network analysis. For example, the introduction of domesticated animal hosts into tick-vertebrate networks increased disease transmission throughout the community, as domesticated hosts acted as super-spreaders, linking nodes that would not otherwise interact [48]. Better understanding of vector-host interactions could also help prevent resurgence in infection from an unknown reservoir. Localised elimination of malaria in Guiana was followed by an increase in rice farming and subsequent displacement of cattle; as a result the malarial vector *Anopheles aquasalis* swapped from livestock hosts to the more abundant humans, leading to a localised outbreak of malaria [49]. Marked consequences for transmission are expected if



vector species or disease-vulnerable host species occupying a central position within a network are added or removed [12,17]. Moreover, certain vectors may act as a **hub species**, with many strong connections to other species both in the same and separated habitats, or **connector species**, that couple spatially or temporally distinct communities, potentially facilitating between-habitat disease transmission [43,50–53]. Such effects could be predicted if well-resolved weighted network data were available: network ecologists increasingly use such data to inform models predicting the occurrence and consequences of **re-wiring** events (where network interactions reconfigure as species are added or lost) [53,54].

**Bridge vectors** between humans and other animals could facilitate sustained transmission from a reservoir host to humans [55],[56]. Biting insect species that routinely interact with multiple well-connected hosts, particularly where individuals contain blood from multiple susceptible host species, are candidate bridge vectors [57]. The incidence and identity of mixed blood meals, where molecular analysis indicates a single insect contains blood meals from multiple host species, is highly relevant for predicting spillover events. However, mixed blood meals are rarely reported, either because the molecular methods used are unsuitable to detect them (**Box 1**), or because they are genuinely rare: only 10 (15%) of the papers included in our network compilation report mixed blood meals. When reported, the frequency of mixed blood meals varied, averaging 13.2% of total blood meals (range: 1% to 34.4%). Using molecular methods that detect mixed blood meals should be a priority (**Box 2**), and could ultimately allow network approaches to be applied on an individual basis, where nodes represent host or insect individuals, and edges represent feeding events that link them[58,59]. Analysis of such networks can reveal vector individuals that have interacted with multiple hosts, highlighting potential disease transmission events.

Finally, quantitative (weighted) networks can reveal potential emerging vectors which occur at low abundance, but which might increase rapidly following a disturbance or environmental change such as habitat degradation or climate change [22], or competitive release following reduction in the abundance of another species [60]. In our network, 48.7% (n=129) of insect species had 10 or fewer blood meal records. Of these species, 61 (47.3%) are known vectors of human diseases, and 32 (24.8%) were recorded interacting with humans. The sparse data for these biting insect species is likely a consequence of their relative rarity as well as sampling methods or locations that underestimate their abundance (Box 2). Nonetheless, they may represent unrecognised sources of cross-species disease transmission, now or in the future. Identification of host use and interaction frequency of understudied vectors is therefore of particular importance for predicting current and future disease transmission risk, and for informing control measures to minimise potential outbreaks [61].

## **Concluding remarks**

Available blood meal interaction data, compiled from a set of global studies, reveal the global pattern of insect-host community structure, and demonstrate the extent to which humans share potential vectors with other vertebrates. Many of the benefits of a network perspective that we have discussed will be best realised with dedicated biting insect-host community-level sampling on a local or regional scale. We encourage researchers to collect such data in a way that minimises biases to facilitate network-based analyses (**Box 2**). The resulting data will allow researchers to address a wide variety of unanswered questions

relevant to understanding and predicting disease transmission and dynamics (see **Outstanding Questions**), both for diseases of humans and more generally.

In the long-term, increasingly reliable sequencing techniques such as shotgun metagenomics [62] will offer unique opportunities to gather large-scale, long-term network data sets. Ultimately, these could allow global-scale biomonitoring to detect changes in community structure and identify emerging disease threats [63][64].

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451

## 452 Glossary

453 **Bridge vector:** A vector that acquires the causative agent of a disease from an infected reservoir  
454 host and transmits it to a human or secondary host.

455 **Competent vector:** An organism with the capacity to maintain and transmit a particular infectious  
456 agent, or with the ability to generate a new infection when interacting with a susceptible host.

457 **Connectance:** A network metric indicating the fraction of potential links that are realised,  
458 calculated as  $L/HP$ , where  $L$  is the number of links and  $H$  and  $P$  are the number of host and parasite  
459 nodes, respectively.

460 **Connector species:** Species in a network that link groups or habitats together, typically through  
461 high mobility or varied interactions that cross structural, temporal or behavioural boundaries.

462 **Degree centrality:** The number of links a species has in a network.

463 **Disease spillover:** Transmission of a pathogen from its reservoir host into a susceptible population  
464 of an alternative host.

465 **Edge:** A relationship or connection between nodes within a network; synonymous with link.

466 **Epizootic:** A disease outbreak event within a nonhuman animal population, analogous to an  
467 epidemic within a human population.

468 **Hub species:** Species with strong connections to many other species in a network.

469 **Node:** The components of a network. In the context of ecological interactions, species, populations  
470 or individuals can be represented as nodes.

471 **Reservoir host:** An epidemiologically connected population in which a pathogen may be  
472 permanently maintained, and from which an infectious agent may be transmitted to a susceptible  
473 population.

474     **Re-wiring:** The reassembling of interactions within a network, typically as occurs following a  
475     perturbation to the community.

476

**Box 1. Integrating molecular approaches to determine interactions and pathways of disease transmission**

Documenting potential and realised pathways of insect-vectored disease transmission has, until recently, relied on expensive and labour-intensive techniques that are error-prone and often require prior identification of candidate hosts and host specific anti-sera [65–67]. Recent developments in molecular methods can overcome these limitations, generating data on feeding interactions more rapidly and comprehensively, and enabling the identification of multiple hosts from mixed blood meals [68].

Previously, species interactions were commonly identified from antibodies present in insect blood meals using enzyme-linked immunosorbent assays (ELISA) [69,70] and monoclonal antibodies [71]. These methods have now largely been superseded by DNA barcoding, diagnostic PCR (polymerase chain reaction) [69,72–74] and, most recently, metabarcoding. In metabarcoding, group-specific PCR primers are coupled with high-throughput sequencing to identify a broad range of taxa within bulk mixtures [72,75]. While the primary motivation behind the shift to metabarcoding has been to identify hosts of individual vectors, this approach also allows the construction of quantitative networks of host-biting interactions at a scale and resolution that was previously impossible. As the cost of metabarcoding continues to decrease, molecular analysis of blood meals offers excellent opportunities to identify and quantify previously cryptic species interactions. In future, shotgun sequencing holds considerable potential for blood meal analyses, with the advantage that it limits the negative effects of primer bias [62],

although uptake of this approach is currently limited by high costs and a lack of genome data for many species.

478

## **Box 2. Improving the resolution and utility of host-biting networks**

The network in Figure 1 represents an *ad hoc* compilation of published data. While it reveals how host-biting networks are interconnected and their potential applications in disease biology, a full realisation of the sorts of applications we suggest in the main text will require a more targeted approach to generate unbiased data on interactions at a local or regional scale.

**Variation in trapping success for different insect taxa** [76] will result in their uneven representation within networks. In particular, collection methods that use live hosts, such as human landing catches [77], will be heavily biased [78]. Widely-used and efficient insect sampling methods include USA Centre for Disease Control (CDC) miniature light traps and BGS traps with CO<sub>2</sub> bait, which capture a wide range of mosquitos [79], sand flies [80] and biting midges [81,82]. We recommend using a combination of sampling methods to maximise representation of the biting insect fauna. Biases will inevitably remain, however; for example, light traps will under-sample insects with diurnal and crepuscular activity. “Non-attractive” methods, such as malaise traps or suction sampling, will be a valuable supplement to attractive traps, potentially documenting a wider range of biting insects and providing less-biased data on their relative abundances [83]. A common approach to construct weighted interaction is to combine randomised sampling (to document abundances in an unbiased way) with more targeted sampling of component species (to generate data on interaction frequencies efficiently)[84,85].



**The spatial location of sampling** will influence both the species composition of biting insects, and the set of potential hosts with which they are interacting. For example, samples taken within or close to human habitation will over-represent both anthropophilic insects and human blood meals [27]. Humans are likely to be exposed to a much wider variety of biting insects (and will be connected indirectly to a much wider variety of their alternative hosts) when they spend time outside the domestic setting, for example, for farming or recreation [86,87]. Non-urban settings are also prime contexts for the emergence of novel and bridge vectors as well as novel pathogens, and should therefore be a priority for network-focused sampling.

The choice of **molecular analysis methods** – especially the use of species-specific techniques and primer sets - can lead to incomplete or biased blood meal identification [88]. Metabarcoding methods are preferable, since they are likely to document a wider set of potential hosts including many that may not have been recorded previously. Universal primer sets that are validated using *in silico* analysis, combined with high-throughput sequencing, can accurately and rigorously characterise the species composition of blood meals and generate unbiased interaction data [74].

Limited **mixed blood meal** reporting in the literature may reflect methods of blood meal analysis that are not appropriate for their detection and identification [89,90]. Wherever possible, metabarcoding methods should be used to screen samples to detect and report mixed blood meals, facilitating the identification of bridge vectors and informing disease transmission risks [74].

## Figure Legends

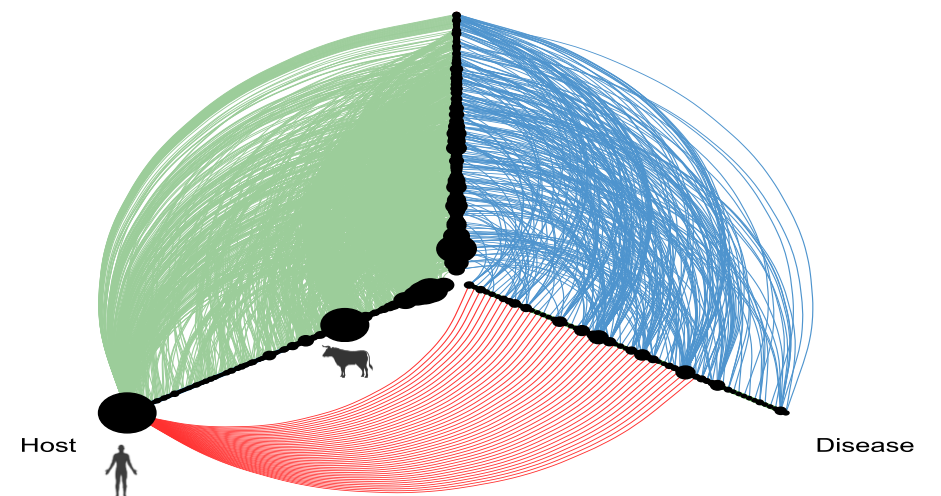
### Figure 1. Host-biting networks and their associated disease transmission pathways.

Bottom: Global bipartite [91] network of host-biting interactions, highlighting how humans (red node and edges) are embedded into the community. Node and edge widths are proportional to frequency. Nodes are resolved to species for hosts and genus for biting-insects. Middle: Bipartite network linking disease (upper) to vectors (lower). Data on disease-vector interactions were extracted from Vectorbase [19]. Red links represent diseases that are transmissible to humans. Mosquito genera (*Aedes*, *Culex* and *Ochlerotatus*) vector the largest number of human disease (23, 18 and 15 respectively). Top: Hive plot [92] showing how hosts (left axis), biting insects (vertical axis) and their respective diseases (right axis), are globally interconnected. Interactions between hosts and biting Diptera are represented in green, vector-disease links in blue, and diseases that are transmissible to humans in red. Nodes along each axis scale with frequency. Taxon silhouettes are from BioRender.com. Data were compiled from published literature on blood meals of biting Diptera. To maximise the amount of relevant data, source publications were identified using *ad hoc* searches in Web of Science and Google Scholar with search terms including 'blood meal', 'mosquito ecology', 'metabarcoding', 'vector-host', and genus, family and subfamily names for biting insect taxa. We also scrutinised the citations within these publications to identify additional relevant literature. Data were extracted from peer-reviewed articles that conducted molecular blood meal analysis for >1 biting Diptera species. To maximise our data set, we included data generated using a variety of blood meal analysis methods including diagnostic PCR approaches using species-specific or general vertebrate primers, ELISA, gel diffusion immunoassays, precipitin tests, microsatellite analyses, and monoclonal antibodies.

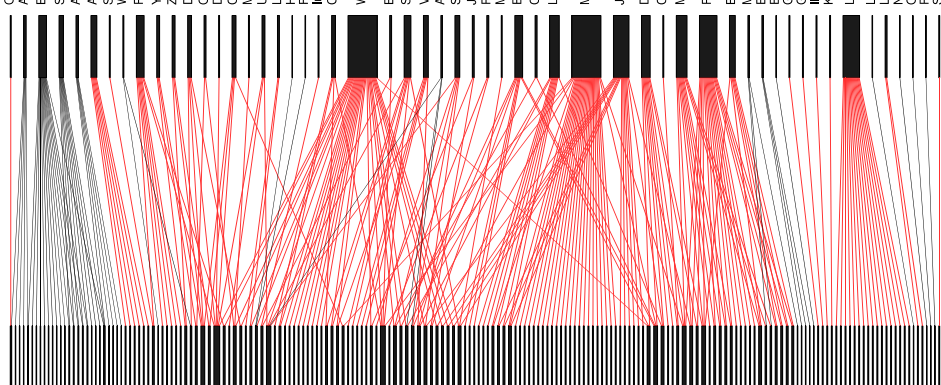
504

505 **Figure 2. Smoothed accumulation and extrapolation curves to assess the completeness of**  
506 **sampling.** (a) Number of host species recorded for the five most frequent biting Diptera  
507 species in the compiled dataset, as a function of sampling effort (the number of blood meals  
508 screened). (b) Total numbers of hosts, biting insects and interactions recorded in the whole  
509 dataset, as a function of sampling effort (the number of blood meals screened). These  
510 curves suggest that the level of sampling achieved across studies is sufficient to record most  
511 biting Diptera (red) and host (blue) species, but that many interactions among these species  
512 remain undocumented. Curves were calculated and plotted using the R package iNEXT [93].  
513 Shaded areas show 95% confidence intervals for the predicted values.

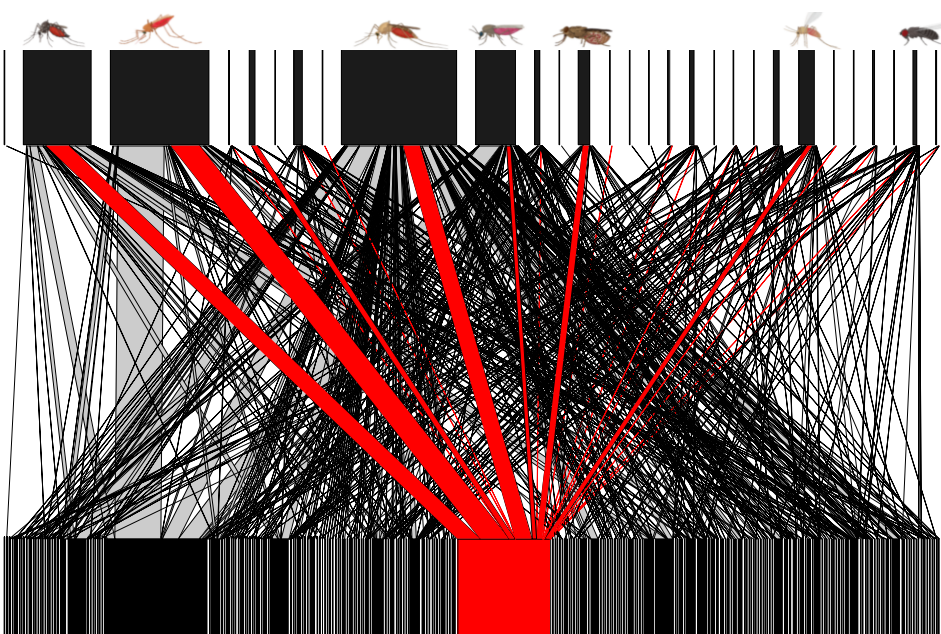
# Biting Insects



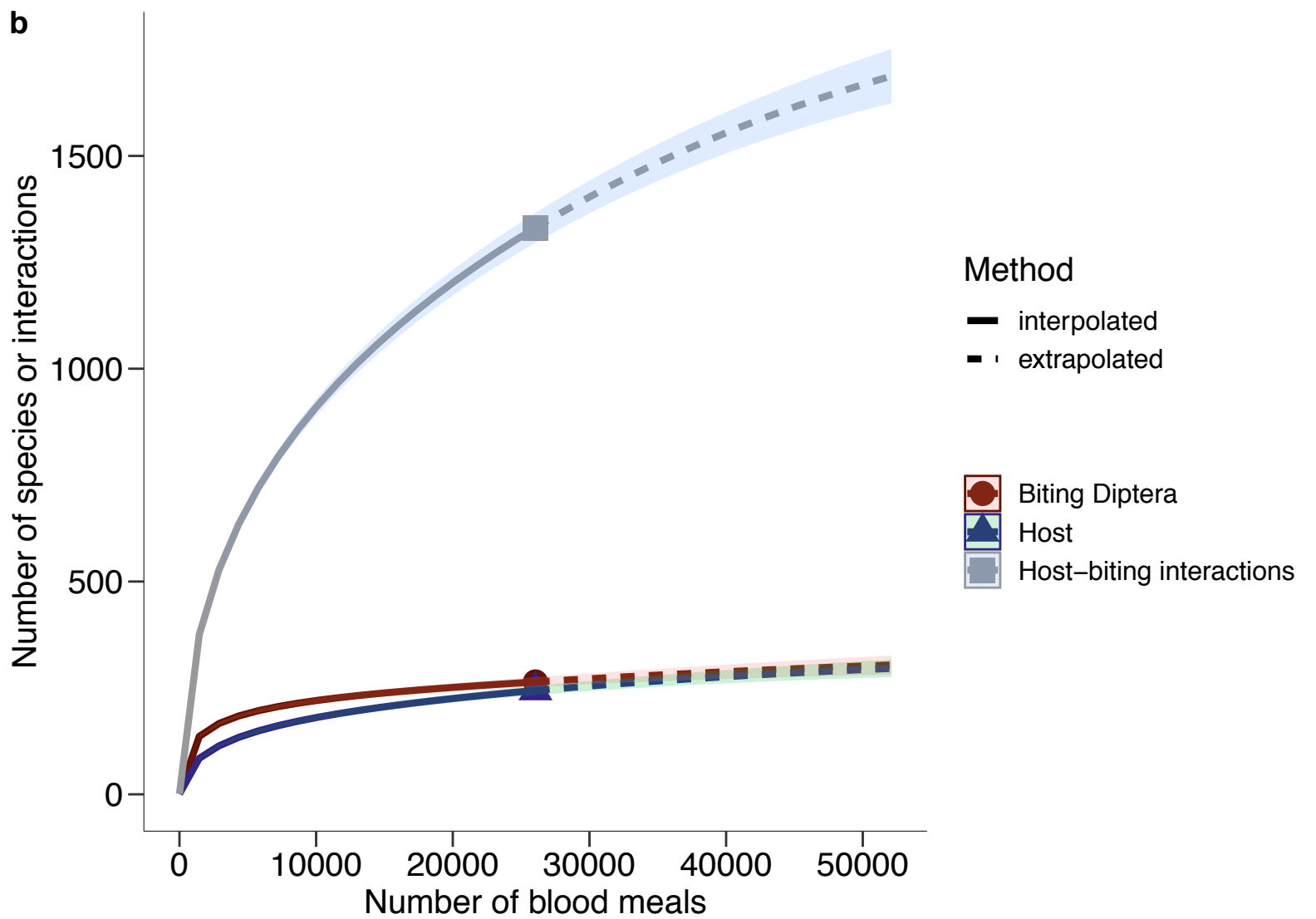
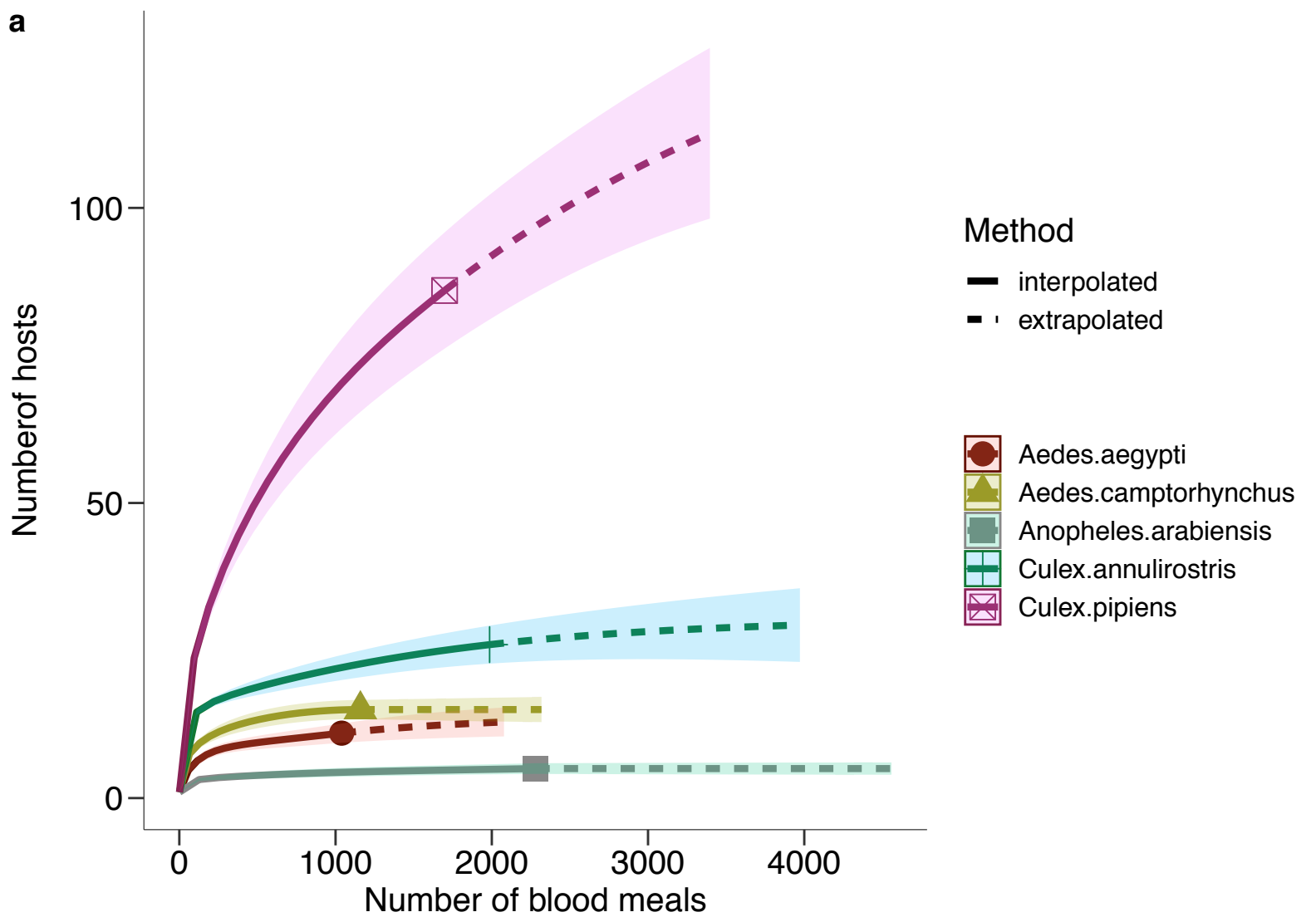
Onchocercidae disease  
 Avian malaria (Haemoproteus)  
 Bluetongue disease  
 Schmallenberg disease  
 African horse sickness  
 African trypanosomiasis  
 Spondweni fever  
 Wolbachia  
 Rift Valley fever  
 Yellow fever  
 Zika virus disease  
 Dengue fever  
 Oropouche virus disease  
 Chikungunya  
 Mayaro virus disease  
 Usutu virus disease  
 La Crosse encephalitis  
 Hantaan virus disease  
 Frog erythrocytic virus disease  
 Inkoo virus disease  
 California encephalitis  
 West Nile fever  
 Batai virus disease  
 Sindbis fever  
 Venezuelan equine encephalitis  
 Avian malaria (Plasmodium)  
 Saint Louis encephalitis  
 Jamestown Canyon encephalitis  
 Pocomo viral encephalitis  
 Murray Valley Encephalitis  
 Eastern equine encephalitis  
 Cache Valley virus disease  
 Lymphatic filariasis  
 Malaria  
 Japanese encephalitis  
 Dirofilariasis (Dirofilaria immitis)  
 Catu virus disease  
 Murray Valley Encephalitis  
 Ross River fever  
 Barmah Forest Virus disease  
 Myxomatosis  
 Epizootic hemorrhagic disease  
 Equine encephalosis  
 Gambia virus disease  
 Illia virus disease  
 Keystone virus disease  
 Leishmaniasis  
 Leucocytozoonosis  
 Lolasia  
 Nagana  
 Onchocerciasis(Onchocerca cervicalis)  
 Rodent malaria  
 Stratford virus disease



*Aedes* *Anopheles* *Culex* *Culicoides* *Glossina* *Phlebotomus* *Simulium*



*Bos taurus* *Homo sapiens*



## 1 Outstanding Questions

- 2 • What factors predict host-biting interactions (e.g. phylogeny, traits, host abundance,  
3 abiotic factors), and can they be used to predict interactions for under-studied  
4 vectors?
- 5 • What influences the apparent host-specialisation of biting insects, and to what  
6 extent is this determined by encounter rates with hosts?
- 7 • Can network data be used to predict changes in biting frequency (and disease  
8 transmission) when the densities of hosts that share potential vectors change?
- 9 • How common are mixed blood meals, and is their frequency correlated with the  
10 degree centrality of individual biting insect species?
- 11 • To what extent does the frequency that a pair of hosts is detected in mixed blood  
12 meals match the biting frequencies on those hosts inferred from interaction  
13 networks?
- 14 • How does the structure of host-biting networks change seasonally, geographically,  
15 and across environmental gradients?
- 16 • Are there biting insect species that consistently fill the role of hub and connector  
17 nodes across different geographical locations?

18