

## Exploring the Mosquito–Arbovirus Network: A Survey of Vector Competence Experiments

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**Abstract.** Arboviruses receive heightened research attention during major outbreaks or when they cause unusual or severe clinical disease, but they are otherwise undercharacterized. Global change is also accelerating the emergence and spread of arboviral diseases, leading to time-sensitive questions about potential interactions between viruses and novel vectors. Vector competence experiments help determine the susceptibility of certain arthropods to a given arbovirus, but these experiments are often conducted in real time during outbreaks, rather than with preparedness in mind. We conducted a systematic review of reported mosquito–arbovirus competence experiments, screening 570 abstracts to arrive at 265 studies testing *in vivo* arboviral competence. We found that more than 90% of potential mosquito–virus combinations are untested in experimental settings and that entire regions and their corresponding vectors and viruses are undersampled. These knowledge gaps stymie outbreak response and limit attempts to both build and validate predictive models of the vector–virus network.

### INTRODUCTION

Arthropod-borne (arbo-) viruses face evolutionary pressures that favor generalism in the range of both vertebrate hosts and arthropod vectors that they can use (reviewed in detail in Ciota and Kramer<sup>1</sup>). That flexibility can pose a particular problem for public health, as it both enables their spread into new locations and ecosystems and adds a layer of unpredictability to their dynamics upon arrival. Experimental studies simplify real-world complexities of transmission and can be used to test not only the basic compatibility of a given virus and arthropod vector species but also vector competence—the relative ability of arthropod vectors to be infected by a virus and then disseminate and transmit it to a susceptible host.<sup>2</sup> Despite arboviruses' evolutionary tendencies toward broad host and vector range,<sup>1,3,4</sup> there are complex genetic underpinnings that govern vector competence,<sup>5</sup> which can manifest as variation in competence between closely related species of the vector<sup>6</sup> or even among populations of the same species.<sup>7</sup>

Vector competence experiments are often conducted in response to the emergence of novel pathogens or the emergence of a known pathogen in a new location with previously untested vectors. The distributions of both mosquito vectors and the viruses they transmit are increasingly in a state of disequilibrium as a result of climate change, global travel and trade, and biotic homogenization.<sup>8,9</sup> Operating in a responsive paradigm, medical entomology is increasingly struggling to keep pace with these shifts, as resources are often abruptly diverted to new study systems to answer questions that support outbreak response and influxes of funding to support such studies are typically reactionary to emergence events.<sup>10</sup>

The patchwork of experimental research efforts to date represents the cumulative history of these moments rather than a systematic exploration of the mosquito–arbovirus network, limiting outbreak preparedness and particularly complicating efforts to predict unrealized links in that network using machine learning.<sup>11,12</sup> As there are no standardized repositories that register vector competence experiments or immortalize their findings, it is currently difficult to evaluate the distribution of research efforts so far and identify important gaps that may be relevant to future outbreaks.

Here, we conducted a systematic review of the mosquito–arbovirus literature using keywords associated with vector competence and screened studies in the Web of Science to identify these studies. Our objective was to determine the taxonomic and geographic patterns in these studies and to identify historical trends driving research in this subfield.

### MATERIALS AND METHODS

A systematic literature search was conducted on the Web of Science to identify suitable records that described vector competence experiments with mosquito-borne viruses. Our search used the following terms: (“vector competence” OR “extrinsic incubation period” OR “vectorial capacity” OR “dissemination”) AND (arbovirus OR virus) AND (experiment\* OR trial OR captive\* OR laboratory) AND mosquito.” Our search in the Web of Science Core Collection via University of Edinburgh institutional access returned 570 records in February 2021.

We performed an initial screen of records based on abstracts, excluding reviews, methodology descriptions, and studies with no experimental infection ( $N = 135$ ), studies with non-virus infections, insect-specific infections, infection regimes with confounding treatments (i.e., coinfection with *Wolbachia* or insect-specific viruses;  $N = 71$ ), studies involving infection in non-mosquitoes, experimental vector infection without any reported results describing competence quantitatively or *ex vivo* data ( $N = 55$ ), and studies fitting multiple exclusion criteria ( $N = 22$ ). For the remaining 287

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records, we undertook a second round of screening to determine suitability (i.e., did the study include some sort of experimental test of mosquito competence for arbovirus transmission, such as infectious bloodmeal, intrathoracic injection, or feeding on viremic animals). After excluding one study for which we could not access the full text,<sup>13</sup> we were left with 265 studies that were within the scope (see Supplemental Table 1). For each of these, we recorded the species pairs of mosquitoes and arthropod-borne viruses that were experimentally tested together, regardless of how competence was measured (including both measurements of virus [infectious virus versus viral RNA] and mosquito tissues analyzed [e.g., body, legs, head]) or whether the vector was found to be competent or not; the country from which wild mosquitoes were originally collected (even if populations were maintained in laboratory settings long-term); and when available, mosquito subspecies and (as applicable) dengue virus serotype. Using the taxize R package,<sup>14,15</sup> we updated mosquito binomial names if a more recent valid name could be found in the National Center for Biotechnology Information taxonomic database; in all other cases, we used taxonomy reported verbatim from source studies, including nonstandard naming conventions (e.g., "Culex sp.", "Culex declarator/mollis").

All analyses were conducted and figures generated in R software version 4.0.3.

## RESULTS

**Taxonomic coverage.** We found a total of 298 pairs of viruses ( $N = 35$ ) and mosquito species ( $N = 122$ ) that have been tested experimentally, leaving the majority of all possible pairings of these specific viruses and mosquitoes untested (93%; 3,972 of 4,270 possible pairs; Figure 1). Among these, some viruses were entirely untested in *Aedes* or *Culex* vectors (seven and six, respectively), and most were untested in *Anopheles* (27 viruses) (Figure 2). Although some of these pairings may not be relevant for experimental vector competence testing (e.g., viruses and vectors that do not overlap geographically), there are many untested pairings of potential public health importance, such as Everglades virus in *Aedes aegypti*, that warrant further investigation given ongoing and projected range expansions and changing climatic suitability for transmission.<sup>8,9</sup> However, for several pairings, our search parameters yielded no results in the Web of Science, despite the existence of relevant publications, such as Murray Valley encephalitis virus and yellow fever virus in *Culex* spp.<sup>16–20</sup> or Ross River virus (RRV) and Sindbis virus in *Ae. aegypti*.<sup>21,22</sup>

Even within tested mosquito–virus combinations, effort is distributed unevenly. A small subset of viruses and their main vectors are extremely well studied: the 10 most commonly tested combinations are dengue virus in *Ae. aegypti* ( $N = 47$  studies) and *Aedes albopictus* ( $N = 19$ ), Zika virus in *Ae. aegypti* ( $N = 35$ ) and *Ae. albopictus* ( $N = 18$ ), chikungunya virus in *Ae. aegypti* ( $N = 24$ ) and *Ae. albopictus* ( $N = 23$ ), and West Nile virus in *Culex pipiens* ( $N = 30$ ), *Culex quinquefasciatus* ( $N = 16$ ), and *Culex tarsalis* ( $N = 8$ ). Most research has focused on flaviviruses (Flaviviridae: Flavivirus;  $N = 13$  viruses, 180 studies) and alphaviruses (Togaviridae: Alphavirus;  $N = 11$  viruses, 79 studies), with less focus on bunyaviruses (Bunyavirales:  $N = 8$  viruses, 26 studies), rhabdoviruses (Rhabdoviridae:

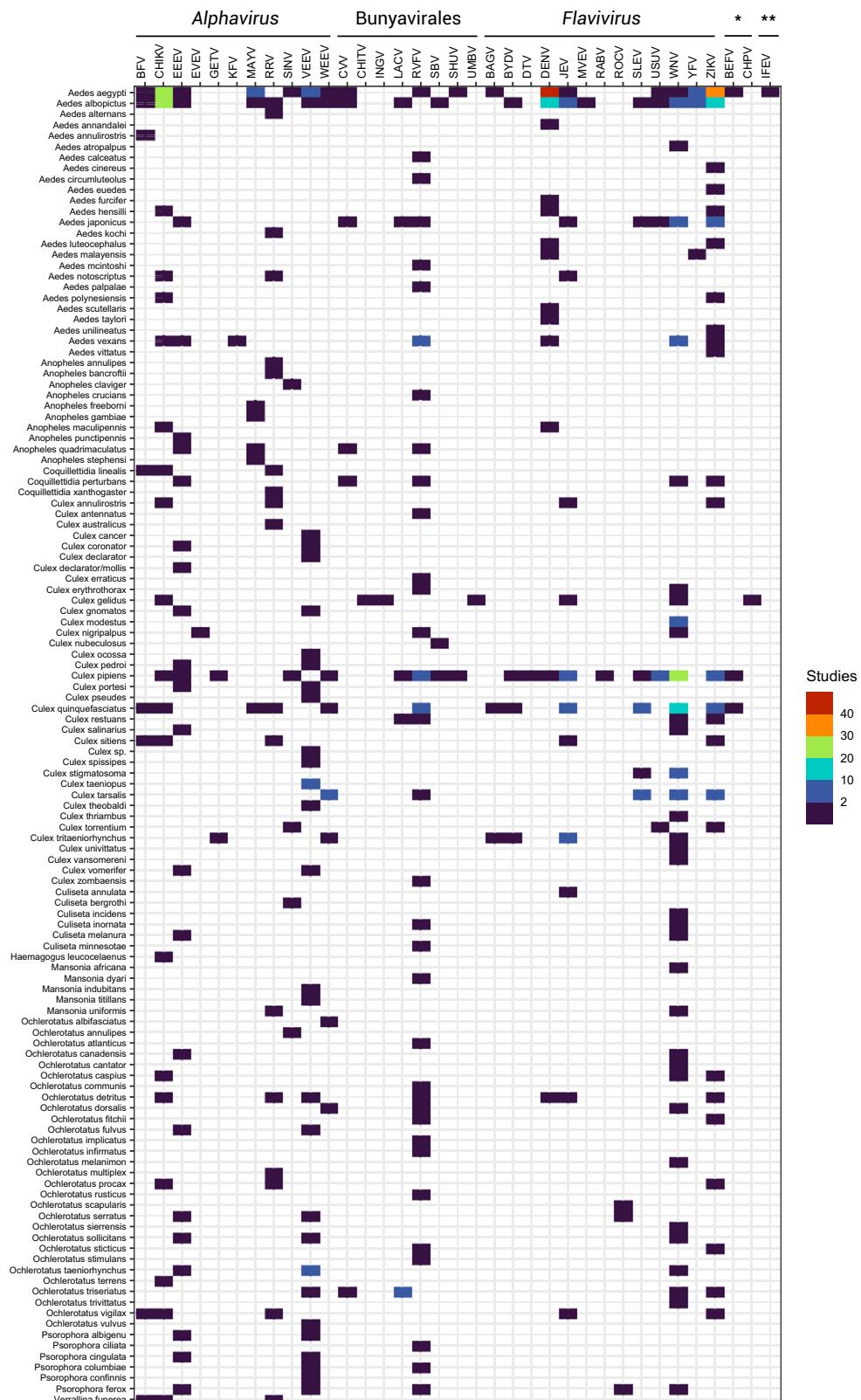
$N = 2$  viruses, 2 studies), and orbiviruses (one study on Ife virus). Even within virus species, distribution of effort was often unequal; for example, dengue serotype 2 (DENV-2), the most readily available lineage for experimental work, is far better studied ( $N = 90$  studies) than the other three serotypes (DENV-1:  $N = 30$ , DENV-3:  $N = 17$ , and DENV-4:  $N = 13$ ). Focus on particular viruses over time tended to track the timing of major outbreaks (Figure 3), including the emergence of West Nile virus (1999–), chikungunya (2013–2014), and Zika virus (2015–2016) in the Americas.

The viruses studied in the widest range of mosquitoes were not always the most-studied viruses overall; the 10 viruses studied in the widest range of mosquito species were Rift Valley fever virus ( $N = 36$  species), West Nile virus ( $N = 36$ ), Venezuelan equine encephalitis virus ( $N = 31$ ), Zika virus ( $N = 26$ ), Eastern equine encephalitis virus ( $N = 24$ ), chikungunya virus ( $N = 21$ ), RRV ( $N = 19$ ), DENV ( $N = 13$ ), Japanese encephalitis virus ( $N = 13$ ), and Barmah Forest virus ( $N = 8$ ).

**Geographic coverage.** Lineage variation in vector competence can be quite striking, even within the geographic range of a single globalized species such as *Ae. aegypti* or *Ae. albopictus*.<sup>34</sup> To explore how arboviral research captures this dimension of natural variation, we recorded where each study's mosquitoes were sourced from. The majority of studies used mosquitoes sourced from the United States (Figure 4), followed distantly by Brazil and Australia. The range of pathogens tested using mosquitoes from a given country is slightly more even, but still the majority of work has focused on the United States, Brazil, Australia, China, India, and western Europe (Figure 5). By both measures, Africa and eastern Europe have been severely understudied, particularly compared with the Americas, where multiple explosive multinational epidemics have forced researchers to answer questions about broad geographic risk.

## DISCUSSION

Our results suggest that research efforts in this field have largely been driven by the shifting priorities of arboviral outbreak response. As such, there are numerous gaps in viral taxonomy, vector taxonomy, and vector geography that limit the utility of available data for future outbreak response. Moreover, the vast majority of mosquito–virus pairs ( $> 90\%$ ) are simply untested within our sample of studies, indicating that network-level understanding of arbovirus ecology is incomplete. In particular, existing descriptions of the mosquito–virus network (e.g., Figure 1 in Evans et al.<sup>11</sup> and Figure 2 in Yee et al.<sup>35</sup>) are challenging to interpret without explicit understanding that the network's architecture is mostly representative of experimental history (Figure 6). In turn, this severely constrains inference about—and prediction of—the true underlying network.<sup>11,35,36</sup> Some gaps may particularly limit ecological inference; for example, anthropophilic vectors such as *Ae. aegypti* are far better studied than bridge and sylvatic vectors such as *Aedes africanus*, *Sabethes* spp., or *Haemagogus* spp. (especially because some of these species (e.g., *Haemagogus leucocelaenus*) have never been colonized, making vector competence experiments especially challenging and reliant on field-collected eggs). These species may be less important during epidemics, but they determine the risk of an enzootic virus



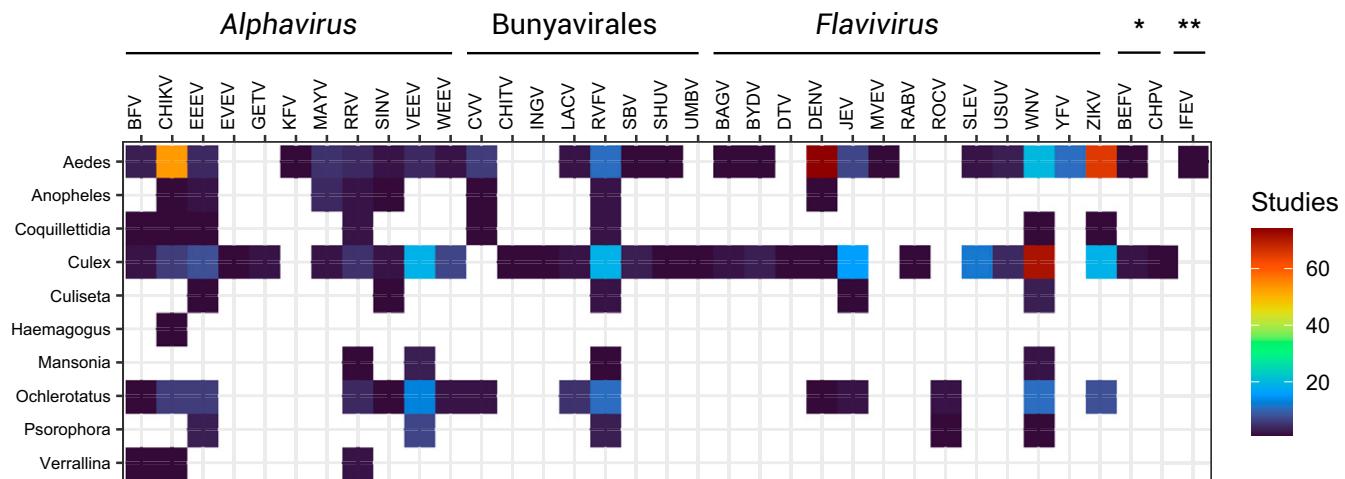


FIGURE 2. Number of studies that have experimentally tested a given arbovirus–mosquito genus pair (as in Figure 1). Abbreviations follow naming conventions in virology. \*Rhabdoviridae; \*\*orbivirus.

reaching humans and, conversely, whether sylvatic cycles are established after an epidemic ends.<sup>35,37</sup>

Efforts to fill these gaps could take several approaches. First, researchers can use network science to identify important vector–virus pairs and conduct vector competence experiments that would fill key knowledge gaps (especially those of likely public health importance). Just as machine

learning can be used to guide species sampling for viral discovery in nature,<sup>38</sup> predictive models can be used to develop or augment shortlists of the vectors that will be most relevant during emergency scenarios that have previously been flagged (e.g., yellow fever establishment in the Asia-Pacific region<sup>39</sup>). The benefits of model-experiment feedback are iterative: at present, many models will struggle with the sparsity of the

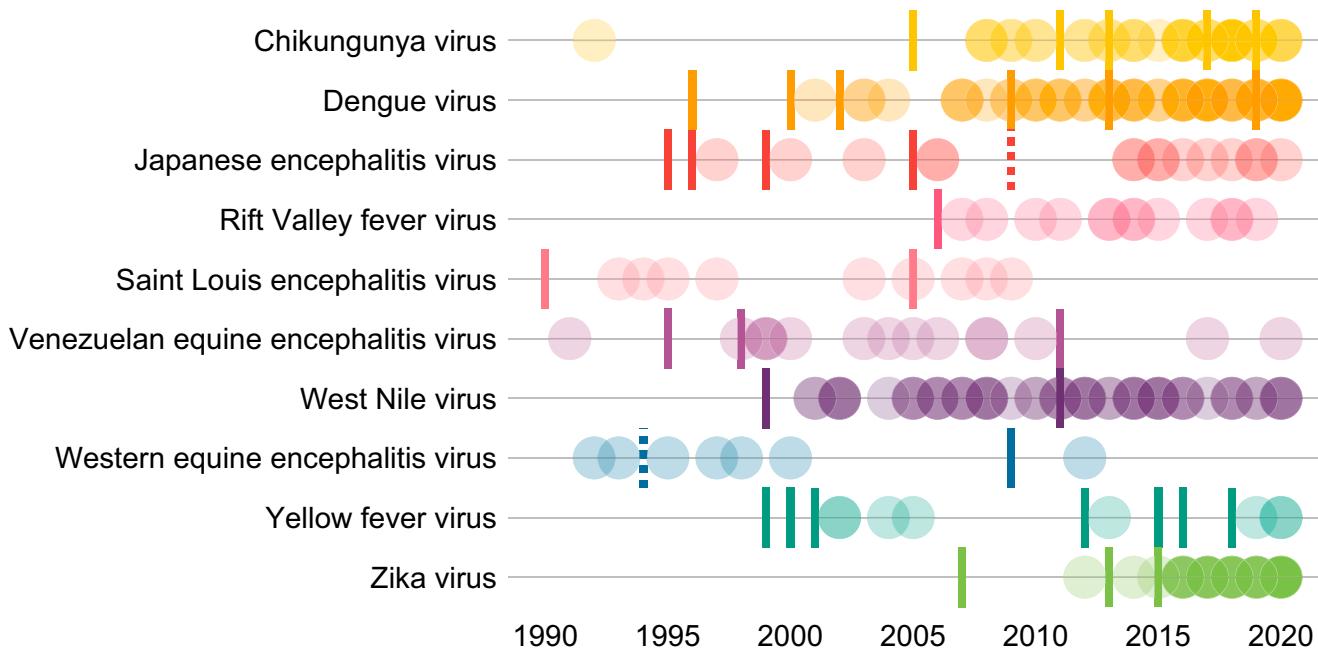


FIGURE 3. Number of studies over time, broken down by the 10 viruses that appeared in the most studies. (Opacity is proportional to the number of studies in any given year.) Tick marks represent notable outbreaks that motivated further inquiry, broken down by pathogen and sourced from primary literature, including the WHO Disease Outbreak News. In some cases, outbreaks for one disease may have increased interest in others (e.g., CHIKV in India in 2005 and ZIKV virus in Yap in 2007 were followed by substantial renewed interest in dengue; the emergence of CHIKV and ZIKV in the Americas in 2013–2015 appear to have been accompanied by incidental research on Japanese encephalitis virus, likely in many of the same experiments). Dashed tick marks indicate other notable developments in virus history. Featured outbreaks include CHIKV: 2005 – India; 2011 – New Caledonia; 2013 – the Americas; 2017 – Italy; 2019 – Republic of the Congo. DENV: 1996 – the Americas; 2000 – DENV-3 introduced to Brazil; 2002 – Brazil; 2009 – the Americas; 2013 – Southeast Asia; 2019 – the Americas. JEV: 1995 – Australia; 1996 – Nepal; 1999 – India; 2005 – India; 2009 – WHO WPRO and SEARO reference laboratories for Japanese encephalitis established (dashed line). RVF: 2006 – East Africa. SLEV: 1990 – Florida; 2005 – Argentina. VEEV: 1995 – Colombia and Venezuela; 1998 – Colombia; 2011 – Colombia and Venezuela. WNV: 1999 – United States; 2011 – Europe and, unrelated, Australia (Kunjin virus). WEEV: 1994 – last known human case in North America (dashed line); 2009 – Uruguay. YFV: 1999 – South America and importation into the United States; 2000 – Guinea; 2001 – Côte d'Ivoire; 2005 – Sudan; 2012 – Sudan; 2015 – Angola and DRC; 2016 – Brazil; 2018 – Brazil. ZIKV: 2007 – Yap Island; 2013 – French Polynesia; 2015 – the Americas.<sup>23–33</sup>

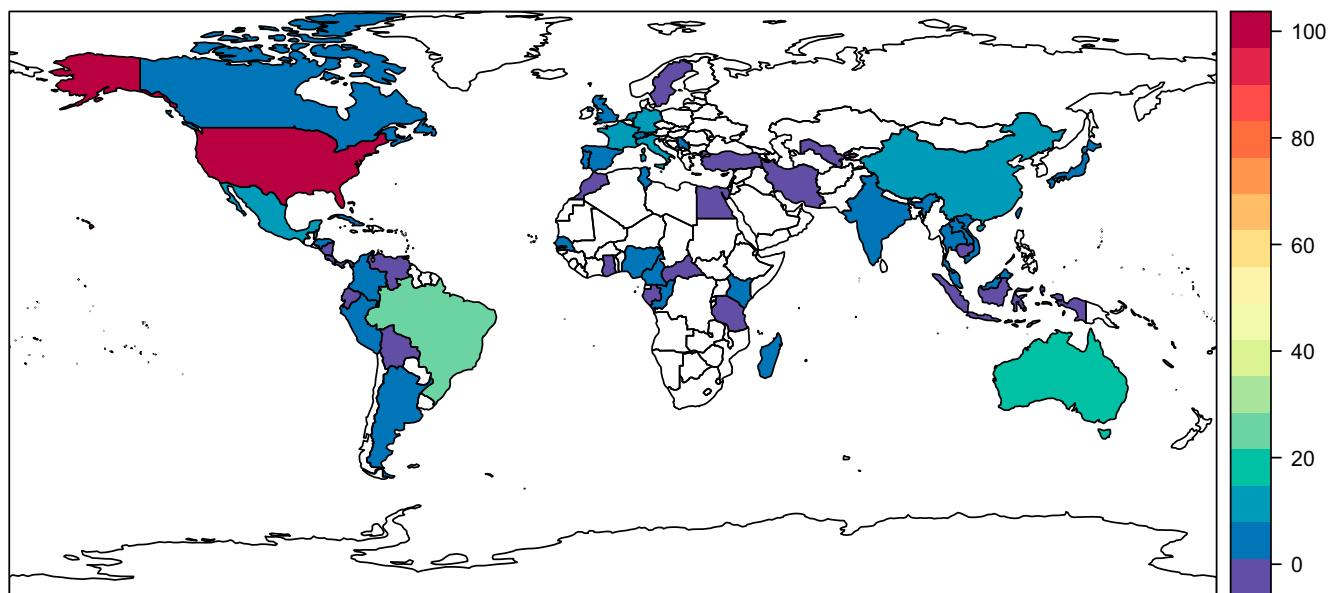


FIGURE 4. Number of studies that used mosquitoes sourced from a given country. Values range from 1 (dark purple) to more than 100 (dark red); countries with no records are left blank.

vector–virus matrix, but targeting important sources of uncertainty will lead to better predictions.

Second, researchers can work toward a more cohesive geographic picture of how risk varies, both for cosmopolitan vectors such as *Ae. aegypti* and *Ae. albopictus* and for vectors that are locally abundant or are known to feed on amplifying hosts.<sup>40</sup> The geographic biases we describe here reflect where a relatively small number of institutions are able to continuously fund and maintain vector colonies. This process is itself often extractive and inefficient in nature (i.e., mosquito colonies established with species from countries facing public health emergencies are often used by researchers in the United States and Europe to generate high-impact publications). This

dynamic can redirect funding from investment in capacity building in the regions where vector-borne disease burdens are highest, perpetuating disparities in both health and scientific research. Deeply linked to capacity and sustainability of institutions housing laboratory colonies, a lack of training in medical entomology has been recently noted as a global issue in the response to outbreaks of vector-borne diseases.<sup>41–43</sup> Investment in these kinds of capacity building in under-supported areas such as Africa, the Middle East, and eastern Europe would be a significant contribution to global preparedness for vector-borne disease outbreaks.

Finally, our study highlights that a substantial breadth and depth of vector competence data are published every year

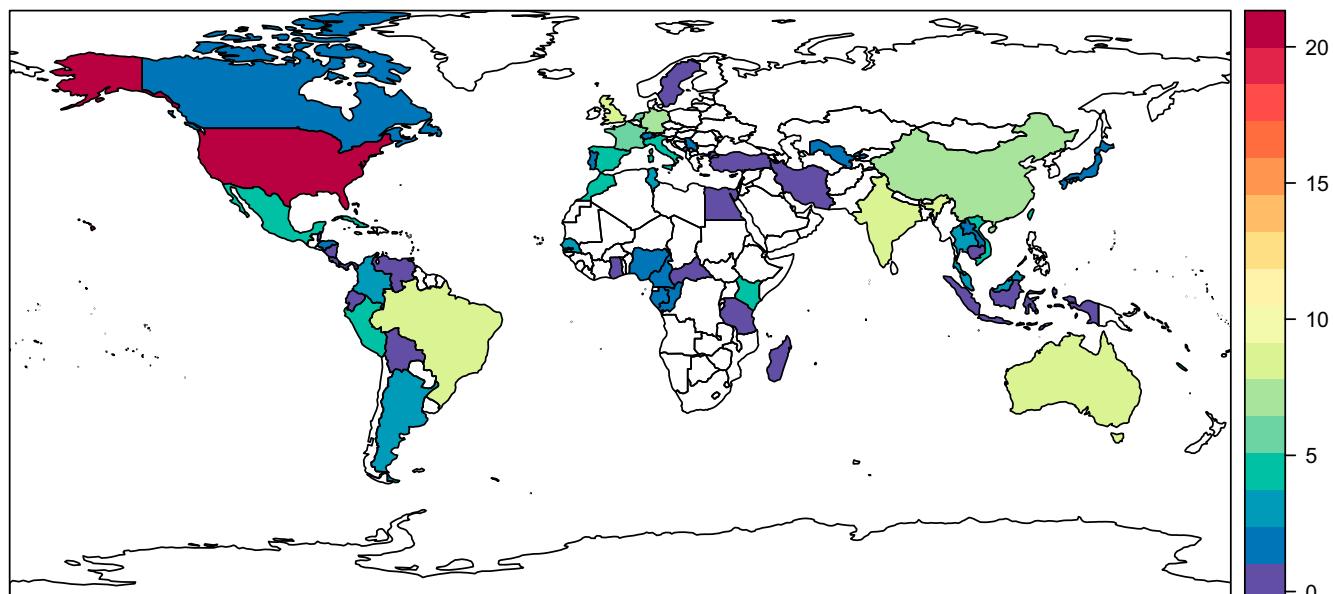


FIGURE 5. The number of pathogens (out of a total of 35 distinct viruses recorded in our review) that has been tested using mosquitoes sourced from a given country. Values range from 1 (dark purple) to more than 100 (dark red); countries with no records are left blank.

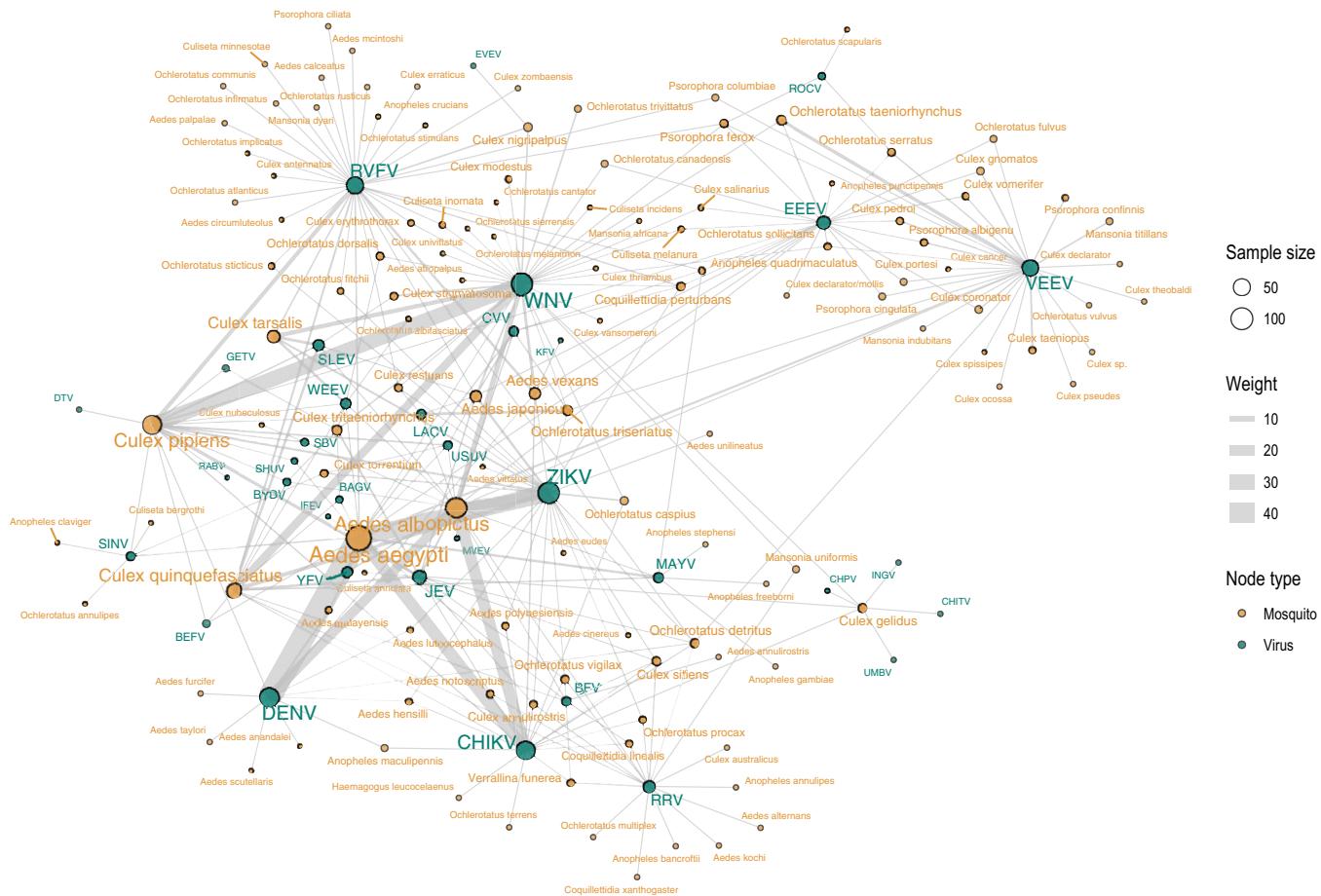


FIGURE 6. Network visualization of arbovirus (green) and mosquito (yellow) pairs experimentally tested. Notably, any observable network of compatible species would be constrained to “fit” inside this sampling-based network, highlighting how experimental effort determines observable pairs of compatible species more than their biology. Abbreviations follow naming conventions in virology. Nodes represent virus and mosquito species, where node size is proportional to the number of studies involving each (range for mosquitoes: 1–136; range for viruses: 1–109). Edges represent a record of an experimentally tested pair, where edge weight is proportion to the number of studies returned for each pair (range: 1–47). Edges do not record species compatibility.

in the peer-reviewed literature, but currently, the results of these experiments have no standardized home. Other recent studies highlight that synthesis of these data is possible, despite the complexity of metadata required to describe variation in experimental protocols<sup>6</sup>; however, our study highlights the challenges of recovering “findable” data from the vector competence literature. Limitations we encountered included 1) Web of Science is not a comprehensive record of this research area, and older studies in particular appear to be missing; 2) keywords we used may not have captured all of the relevant studies because of variable terminology; 3) a handful of non-English-language publications—in particular, Spanish and French language publications from Latin America and Africa, respectively—were not captured by our search terms; and 4) not all studies reported reusable experimental results and metadata. Developing a standardized database of vector competence experiments with direct user contributions—ideally supported by a universal set of minimum data and metadata standards<sup>44</sup>—would help translate the inconsistent and patchy funding in this field into more immortalized data. In doing so, such a database would help researchers identify geographic and taxonomic knowledge gaps further in advance of public health emergencies and

would make more data immediately available to public health agencies in a searchable format once an outbreak begins.

As we show here, data science approaches can be useful to identify trends and gaps in scientific understanding of arboviral ecology and evolution.<sup>12</sup> In some cases, filling these gaps will be more challenging; for example, establishing colonies is harder for some mosquito species than others, and some viruses require higher biosafety levels, limiting the number of researchers with the ability to safely work with them. Future work may also aim to expand our scope to other medically important vectors, including ticks and midges (for which we excluded a handful of studies that were identified by our search terms). Similarly, future work could examine trends in how coinfection dynamics are studied and tested. Both insect-specific viruses and *Wolbachia* have been considered as potential biological countermeasures to arboviral transmission in mosquitoes<sup>45–48</sup>; however, the network of pathogen-coinfection-vector combinations has been characterized even less systematically, and these data remain largely unsynthesized. Addressing these types of questions in the future may point to new opportunities for both empirical research and modeling that harnesses these data to predict and prevent arboviral emergence.

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Raw data and code to reproduce analyses are available on Github at <https://github.com/viralemergence/bolide>.

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