ILLINOIS

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Illinois Natural History Survey
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Surveillance of *Aedes aegypti* and *Aedes albopictus* in the State of Illinois

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Summary

Mosquito communities were actively sampled using a variety of traps in 8 counties in south-eastern Illinois with the intent to detect whether *Aedes albopictus* and *Ae. aegypti* were present in these locations. Specimens collected through routine surveillance and shared by 8 local public health departments or mosquito abatement districts were also identified to species to detect the presence of this species across a wider range of counties. While *Ae. albopictus* was found to be present in all of the 8 counties actively sampled (providing the first records of occurrence for these locations) and for 6 of the counties for which we received collections, the numbers of *Ae. albopictus* collected differed among locations. *Culex* spp. mosquitoes were also caught in the traps. *Aedes aegypti* was not found to be present in these locations. All collected *Aedes* and *Culex* spp. were screened for 6 arboviruses, though no positives were found. By sequencing a segment of the mitochondrial CO1 gene of a subset of female *Ae. albopictus* from 10 locations in Illinois we identified 17 unique genetic sequences (haplotypes), with likely at least 4 distinct genetic lineages being present in Illinois, with different geographic areas having distinct genetic populations. This suggests that the composition of this vector species in Illinois is the result of a complex and dynamic invasion history. Major questions for future work are how these genetic differences relate to the increase in abundance of *Ae. albopictus* in recent years, and whether there are phenotypic differences among these populations that might impact the epidemiology of vector-borne diseases in Illinois.

Background

The worldwide range expansion over recent decades of important mosquito vectors of arthropod-borne viruses (arboviruses), such as *Aedes aegypti*, *Ae. albopictus,* and *Ae. japonicus* poses a serious threat to global health. With their introduction and expansion, these container-breeding species can alter the epidemiology of arboviral diseases through their vectorial capacity for native or independently introduced arboviruses (Gubler 1998, Juliano and Lounibos 2005). Examples include the recent outbreaks of dengue virus (DENV) in South and Central America (Gubler 2002), chikungunya virus (CHKV) in Italy and La Reunion (Ligon 2006, Charrel *et al*. 2007) and Zika virus (ZIKV) in the Pacific and Central and South America (Mlakar *et al*. 2016). Autochthonous transmission in the US has been recorded for all three of these viruses (Bouri *et al*., 2012, CDC 2018, Marini *et al*., 2017). In addition, La Crosse virus (LACV), transmitted by *Ae. japonicus* and *Ae. triseriatus*, has been the major cause of arboviral encephalitis among children in the United States (McJunkin *et al*. 2001, Harris *et al.* 2015). In the case of dengue viruses, an estimated 3.97 billion people globally are at risk of infection (Brady *et al*. 2012). Approximately 500,000 people with severe dengue fever require hospitalization each year, a large proportion of whom are children and about 2.5% of those affected die (WHO 2017a). Chikungunya has been identified in over 60 countries in Asia, Africa, Europe and the Americas, and has caused sporadic outbreaks resulting in significant morbidity (WHO 2017b).

Recent outbreaks of ZIKV infection in Central and South America have further intensified the public concerns about mosquitoes and pathogens transmitted by them. Since its first isolation from a monkey in the Zika forest of Uganda in 1947, human ZIKV infections were sporadically reported in Africa and Asia until the 2007 outbreak in the Yap State, Federated States of Micronesia (Mlakar *et al*. 2016). After the 2013 and 2014 epidemics in French Polynesia, New Caledonia, the Cook Islands, and Easter Island, ZIKV has spread to 33 countries in Central and South America in 2015 (Mlakar *et al.* 2016). In the US, as of November 22, 2017, 5,304 travel-associated and 226 locally acquired ZIKV infection cases have been reported (CDC 2017a). Illinois is accounted for 110 travel-associated cases (CDC 2017a). Most people with ZIKV infection are asymptomatic or they experience only mild symptoms, including fever, rashes, joint pains, and conjunctivitis (CDC 2017b). Serious complications are not common and deaths are rare. However, increasing evidence from recent outbreaks of ZIKV disease in Central and South America suggest that ZIKV infection during pregnancy can result in microcephaly, a condition in which babies are born with an abnormally small head, other congenital anomalies, and miscarriages. Guillain-Barré syndrome also has been associated with recent ZIKV disease (Mlakar *et al*. 2016).

Currently, there are no effective antiviral therapies for arboviruses, and vaccines are only available for a limited number of them. Thus, vector control remains the most effective means of preventing and limiting outbreaks of arboviruses. Ecological data about vector abundance and distribution is critical for targeted intervention. Historically, arbovirus surveillance in Illinois has mainly focused on *Culex* spp. mosquitoes, the vectors of West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) (Flaviviridae: Flavivirus) in urban areas (Ruiz *et al*. 2004, Lampman *et al*. 2006, Harbison *et al.* 2010). However, the presence, abundance and distribution of *Ae. aegypti* and *Ae. albopictus* in Illinois have not been comprehensively investigated. Therefore, we have an urgent need to conduct mosquito surveillance and update

historic maps of *Ae. aegypti* and *Ae. albopictus* distribution patterns in the state. Given the known presence of *Ae. albopictus* in certain parts of Illinois and the relatively frequent introduction of (travel-related) infected human cases, it is also critical to see whether dengue, Zika, or La Crosse viruses can be found in local populations of these highly competent vectors.

To address this gap in our knowledge of the epidemiology of these two vectors in Illinois, we conducted a seasonal survey for *Aedes* mosquitoes in selected residential areas of the state in summer 2017. Specifically, using BG-sentinel and oviposition traps that are designed to attract container-breeding mosquitoes, we surveyed for invasive *Aedes* species in domestic, peri-domestic, and rural areas. Collected specimens were identified to species morphologically and using molecular techniques and were then screened for arboviral infection. To gain more insight into the invasion history and origins of *Ae. albopictus*, we also investigated the population genetics of several local populations. Our aim was to generate ecological, genetic and epidemiological data that can guide the development and deployment of arbovirus infection prevention measures in the State of Illinois.

Methods

Study area

We surveyed larger communities in the south-eastern half of Illinois for *Ae. aegypti* and *Ae. albopictus* during the mosquito breeding season of 2017 (July - October). The studies were conducted in the cities of Danville, Paris, Marshall, Robinson, Lawrenceville, Mt. Carmel, Albion and Fairfield (Table 1). These communities are located along the Illinois Highway IL-1 and IL-15, and located in counties for which there had been no occurrence records of *Ae. albopictus* to date. Additional cities were included when local health departments provided assistance in sampling. Out of 27 local public health departments contacted by the Medical Entomology Laboratory, 17 agreed to assist in sampling. Eight of 17 local public health departments have sent mosquito samples to the Medical Entomology Laboratory at Illinois Natural History Survey. These include the counties of Adams, Jackson, Kane, McLean, Macon, Sangamon, St. Clair and Will.

Mosquito egg, larval and pupal sampling

Guided by officials of local city halls or departments of public health, we identified promising habitats for *Aedes* mosquitoes in urban residential areas in each community. Oviposition traps (1.9 L capacity) were lined with germination paper and filled with a sugar maple infusion. These were placed in sampling locations for 13 consecutive nights and mosquito eggs, larvae and pupae were collected every 2 weeks. Following transportation to the lab, mosquito larvae and pupae were allowed to develop and eclose as adults. The resulting adults were subsequently identified to species by morphological characteristics (Darsie Jr and Ward 1981). Additionally, eggs harvested from the germination papers were hatched and reared to the adult stage to allow for species identification (Darsie Jr and Ward 1981). If

morphological identification was not possible, molecular species identification was carried out (Sanogo et al. 2007, Hill et al. 2008). A total of 168 trappings of mosquito eggs, larvae and pupae were performed throughout the season.

Adult mosquito sampling

Adult mosquitoes in urban sites were collected by using BG-sentinel traps to which dry ice was added as an additional cue. These traps are designed to collect adult mosquitoes attracted to human body odor and carbon dioxide. Three BG-sentinel traps were set for one night every 2-weeks in each city between July 25, 2017 and October 12, 2017. A total of 168 samplings were performed to collect adult mosquitoes throughout the season. The samples were transported to the laboratory in a cool box containing dry ice and were subsequently identified to species on a chill table (Darsie Jr and Ward 1981). For the cities where INHS could not sample, local health departments were asked to sample adult mosquitoes with gravid traps. The collected adults were then frozen and shipped to the INHS for identification.

Fig. 1: image of the BG Sentinel trap (on the ground) to collect adult female mosquitoes and an oviposition container (on the tree) used to collect eggs.

Molecular mosquito species identification

When morphological identification was not possible due to excessive loss of identifying characters, molecular species identification including DNA sequencing for Cytochrome C oxidase I (COI) gene was carried out. Total DNA was extracted from a single mosquito leg of each specimen using the Phire Tissue Direct PCR master mix kit (Thermo Fisher Scientific, Waltham, MA). After running PCR to amplify the COI gene using a primer set, MTFN (5'-GGA TTT GGA AAT TGA TTA GTT CCT T-3') and MTRN (5'-AAA AAT TTT AAT TCC AGT TGG AAC AGC-3'), the resulting amplicons were sequenced by the Keck Center at the University of Illinois at Urbana-Champaign. Each sequence read was BLAST searched at GenBank and the mosquito identification was selected if the sequence match was at least 97%. For a small number of mosquito specimens, we were unable to identify them to species after the sequencing and these were identified at their genus level only.

Molecular diagnosis for arboviruses

For the female *Aedes albopictus* and *Culex* spp. mosquitoes that the Medical Entomology Laboratory collected from 8 cities in the summer of 2017, all were screened for a panel of arboviruses. Given the vector status of *Ae. albopictus* for arboviruses of global concern, these included Zika virus (ZIKV) and Dengue virus serotype 2 (DENV). We also screened these

mosquitoes for viruses whose transmission cycle is likely locally maintained. These included West Nile virus (WNV), Jamestown Canyon virus (JCV), Eastern Equine Encephalitis virus (EEEV), and La Crosse virus (LACV). Up to 50 female adults of each mosquito species were pooled by collection date and by city and tested using RT-PCR. Mosquito samples provided by local public health departments were also pooled up to 50 individuals, by collection date, and tested for the same set of arboviruses.

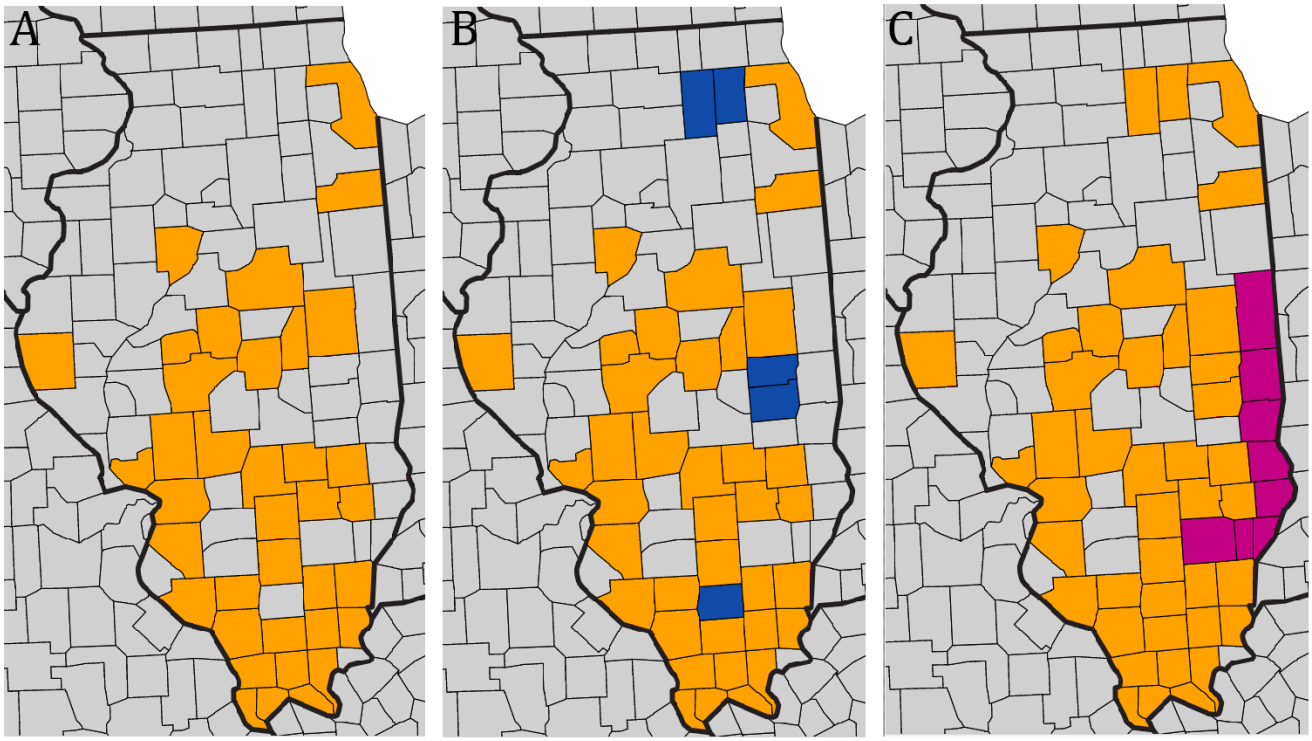

Fig. 2: Occurrence records of *Aedes albopictus* in Illinois. A: Counties with *Aedes albopictus* prior to 2016. B: Counties with blue color that were newly confirmed for the presence of *Aedes albopictus* in 2016. C: Counties with red color that were newly confirmed for the presence of *Aedes albopictus* in 2017.

Aedes albopictus population genetics

To investigate the population genetics of *Ae. albopictus* originating from different cities, a set of specimens were further analyzed for sequence polymorphism of the mitochondrial COI gene. A total of 9 cities were chosen based on the number of female adult *Ae. albopictus* available for the analysis. Only females that were collected as adults in BG Sentinel or gravid traps were used (including samples from oviposition cups would likely increase the relatedness of specimens per location). The list of cities includes Albion, Belleville, Carbondale, Champaign, Decatur, Macomb, Marshall, Mt. Carmel, Paris and Quincy.

DNA was isolated from a leg of each mosquito using the Phire Tissue Direct PCR master mix kit (Thermo Fisher Scientific, Waltham, MA). Obtained DNA was PCR amplified using a MyFi Mix (Bioline USA, Inc., Taunton, MA) and two sets of primers flanking COI gene of *Ae.*

albopictus: 1454F (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and 2160R (5'-TAA ACT TCT GGA TGA CCA AAA AAT CA-3'); 2027F (5'-CCC GTA TTA GCC GGA GCT AT-3') and 2886R (5'-ATG GGG AAA GAA GGA GTT CG-3'). The amplicons from the two PCRs were sequenced separately using primers 2160R and 2027F by Keck Center at the University of Illinois at Urbana-Champaign.

Results

Mosquitoes collected by BG-Sentinel traps

Excluding the mosquitoes identified to genus level only, a total of 24 mosquito species were detected from the survey using BG-Sentinel traps (Table 2). While no *Ae. aegypti* were detected, *Ae. albopictus* was found to be present in all the communities in which the current study was conducted (Table 2; Figure 1). Also, *Ae. vexans*, *Anopheles quadrimaculatus*, *Culex erraticus* and *Cx. pipiens* were detected in all communities surveyed in the current study (Table 2). *Aedes albopictus* was the most abundant mosquito species in Paris, while *Ae. canadensis* was for Danville, Lawrenceville and Robinson, and *Cx. pipiens* was for Mt. Carmel. Nine species were uniquely detected from 6 of the 8 communities surveyed: *Anopheles crucians* in Albion, *Psorophora howardii* and *Uranotaenia sapphirina* in Lawrenceville, *Cx. nigripalpus* and *Ps. ciliata* in Marshall, *Ps. confinnis* and *Ps. horrida* in Mt. Carmel, *Orthopodomyia signifera* in Paris and *Toxorhynchites spp* in Robinson (Table 2).

We performed RT-PCR on all *Aedes* and *Culex* spp. collected during the summer of 2017 to detect WNV, ZIKV, JCV, EEEV, LACV and DENV. No mosquito pool produced positive results.

Mosquitoes collected by oviposition traps

A total of 168 sheets of oviposition paper were collected over 7 sampling trips for 8 cities in Illinois. A total of 12 mosquito species were detected, with *Cx. pipiens* being present in all surveyed communities (Table 3). *Aedes albopictus* was the most abundant species followed by *Cx. pipiens*, as detected by oviposition traps, in all communities except Danville.

Mosquitoes provided by local public health departments

Out of 17 local public health departments (LPHDs) that agreed to participate the current survey, 7 LPHDs sent INHS mosquitoes with unknown identification, while Macon County mosquito abatement district sent INHS all of the *Aedes albopictus* they collected during the survey period (but no other species). *Aedes albopictus* was the most abundant mosquito species in the counties of Adams and Sangamon (Table 4).

Mosquito species diversity and evenness

The highest Shannon mosquito species diversity and evenness was detected in Fairfield, and the least in Paris (Table 5). While we were unable to investigate a direct link between these mosquito biodiversity metrics and arboviral prevalence, due to the lack of positive pools, we did investigate the link between mosquito species richness and abundance (Fig. 3). The relation between total mosquito (all species combined) abundance and species richness by trap site reveals that: in areas with more diverse mosquito communities, the overall abundance of mosquitoes is also greater. This relation is well described by a power function ($y = ax^b$). Interestingly, the same pattern was found when looking at the relation between species richness and *Cx. pipiens* abundance specifically. Greater numbers of *Cx. pipiens* were found in more diverse trap locations, and the relation between these factors was well-described by a similar power function. This indicates that mosquito species richness in an area could predict *Cx. pipiens* abundance. Further work would be required to see whether this metric could be a useful covariate in models of West Nile virus prevalence. For *Ae. albopictus*, however, a different pattern was found, with the highest levels of abundance being associated with locations with moderate levels of species richness.

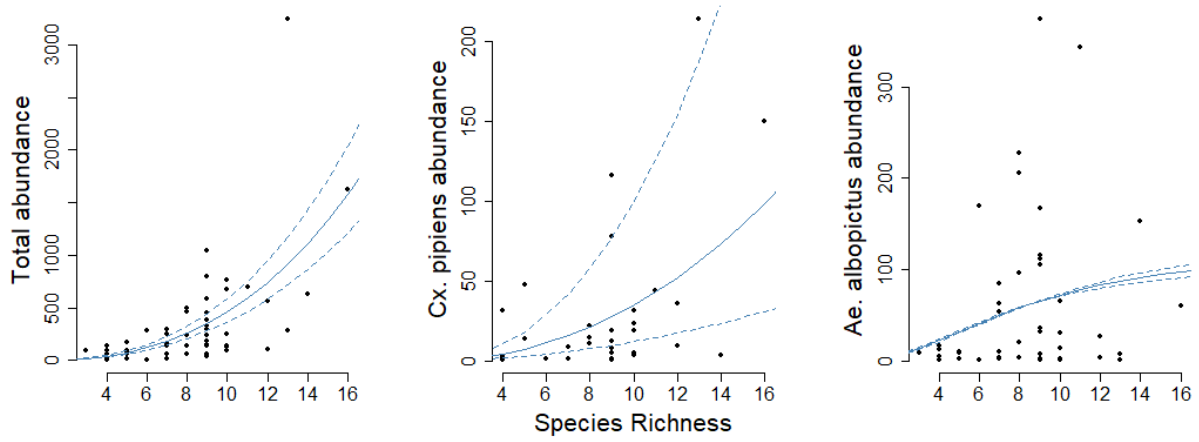

Fig. 3: The relation between mosquito species richness and mosquito abundance. For each trap site, the species richness over the entire season is compared the total mosquito abundance (left panel), the abundance of *Cx. pipiens* (middle panel), and the abundance of *Ae. albopictus* (right panel). The solid blue lines represent the best fit, estimated using maximum likelihood, and the dashed lines the associated 95% confidence interval.

Population genetics

We sequenced a section of 1321 base pairs of the mitochondrial CO1 gene following the methodology of Zhong *et al* (2013). This was done for 245 *Aedes albopictus* females collected from 10 locations across Illinois. The sequences of all individuals were aligned and any nucleotide differences were identified. Overall, 13 sites were found to be variable. Of those, 4 were singletons (i.e., only a single female had a different nucleotide present), while 9 of the sites were parsimony informative (i.e., 2 or more females had this variant). In total, this came to 17 unique sequences, or haplotypes, to which females could be assigned. We investigated the extent of genetic differentiation between populations by calculating F_{st} values using Arlequin (Excoffier & Lischer 2010). These values (Fig. 4) ranged from low levels of differentiation all the way to distinct populations. To test whether the amount of differentiation was related to geographic distance, we investigated isolation by distance (Fig. 5). There was no sign of this (Mantel test, $P = 0.21$), suggesting that the observed genetic differences between populations likely are not due to genetic drift following introduction of an initial founder population. A median joining network

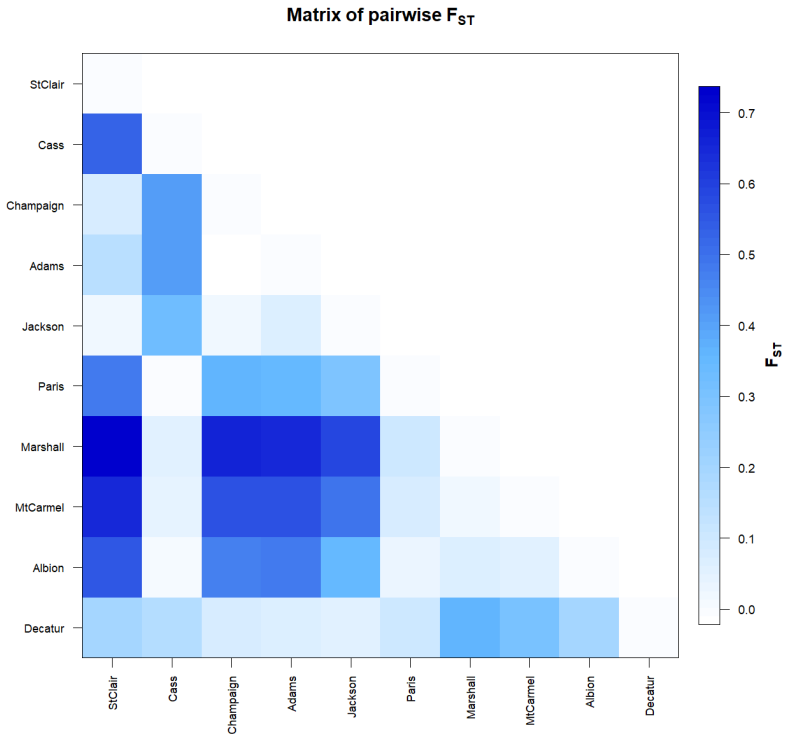

Fig. 4: The amount of structure or differentiation between populations, as indicated by pairwise F_{st} values.

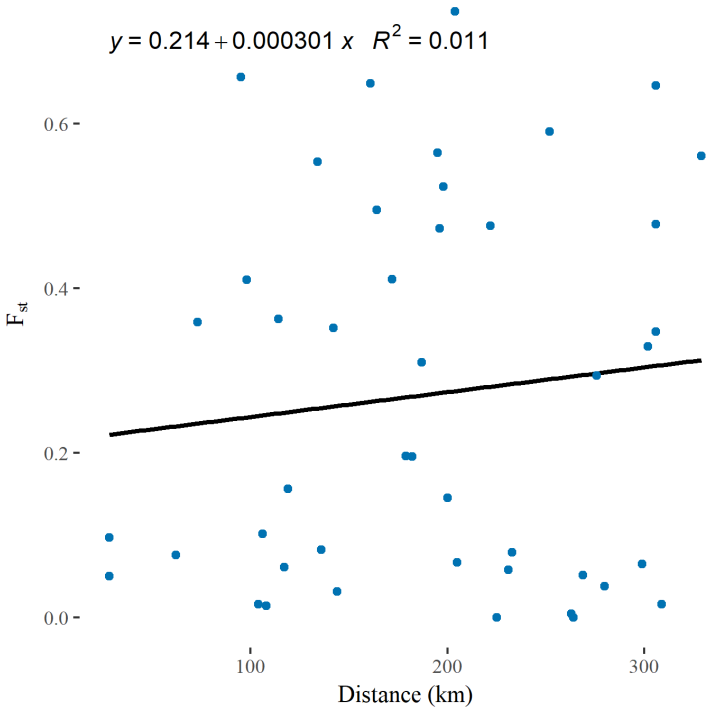

Fig. 5: Pairwise F_{st} values plotted against pairwise geographic distance for all 10 locations

of haplotypes was generated using PopArt (Bandelt *et al* 1999; Leigh & Bryant 2015) (Fig. 6). This likewise suggested there are at least two major clusters of *Ae. albopictus* in Illinois (*Hap_1* and *Hap_2*), each with smaller haplotypes associated with them. We used BLAST searches to look for potential regions of origin of these different haplotypes. When multiple matches were available, we attempted to link these results to the haplotypes identified by Zhong *et al* (2013), who used samples from multiple states and countries. Our *Hap_1* was identical to a haplotype they named h37, which Zhong *et al*. found in populations from Italy, New

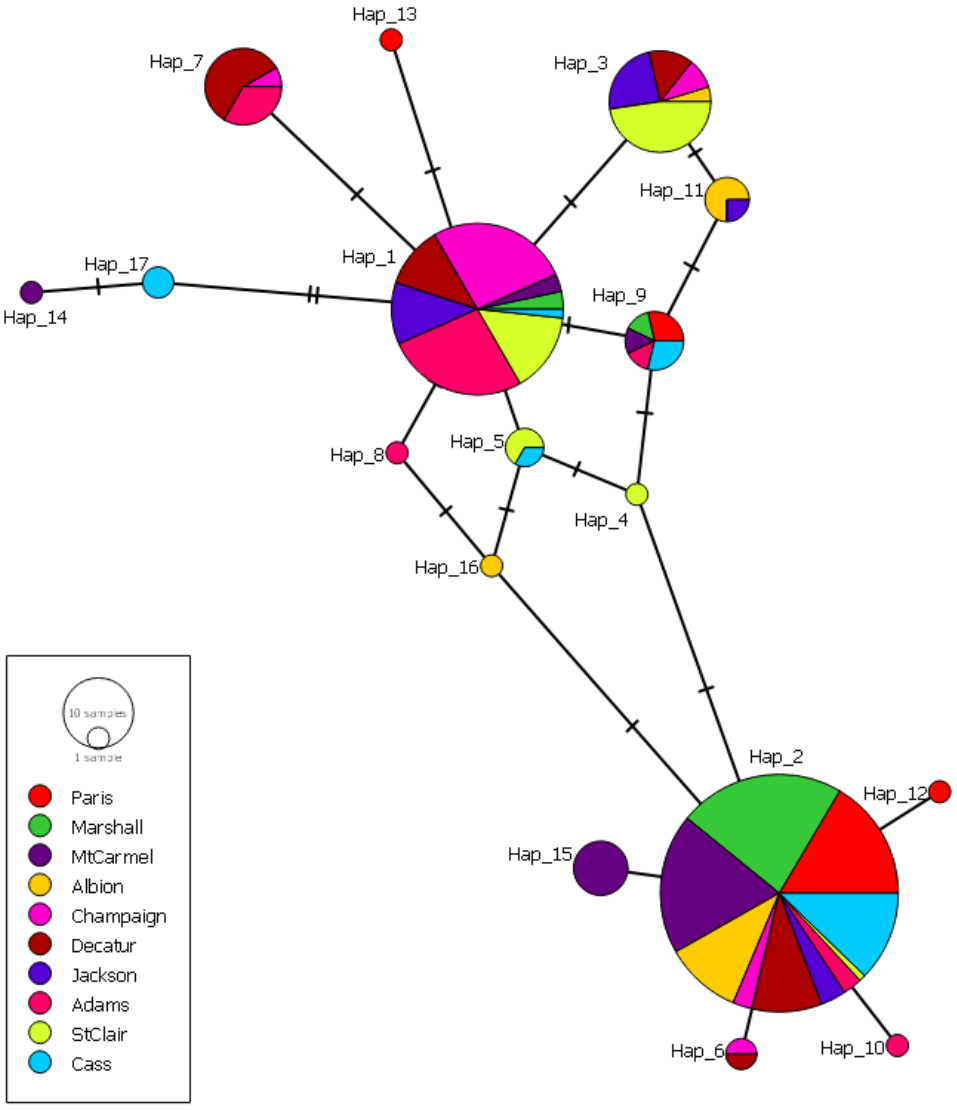

Fig. 6: A haplotype network of *Aedes albopictus* populations in Illinois. Circles represent haplotypes, dashes on lines indicate an additional nucleotide difference between haplotypes. Colors indicate the distribution over locations per haplotype, and size of the circles indicates the number of females with that haplotype.

Jersey, and Texas. Our haplotypes 5, 9, 13, 14 and 17 were 99% identical to h37 as well. The other major haplotype (*Hap_2*) was identical to their h25, which they found in a population from Japan. Haplotypes 4, 6, 10, 12, and 15 (those surrounding *Hap_2* in the network) were 99% identical to h25. Two other haplotypes (*Hap_3* and *Hap_11*) were identical to a haplotype found in Texas, h55, while *Hap_7* was identical to Zhong *et al*.'s h39, which was found in populations from Italy and Texas. Finally, our haplotypes 8 and 16 were similar to their h17 and h3, which they found in populations from Taiwan, Hawaii, and Los Angeles. The distribution over the landscape of the haplotypes we characterized is depicted in Fig. 7. For the major haplotypes (1 and 2) we see a spatial distinction in that *Hap_1* (pink) is more abundant in the western locations and Champaign, whereas *Hap_2* (purple) is the most abundant haplotype in the eastern locations and in Cass county. Of the others, *Hap_7* (green) is present in the more northern locations, and *Hap_3* (blue) in the southernmost locations, as well as in Decatur and Champaign.

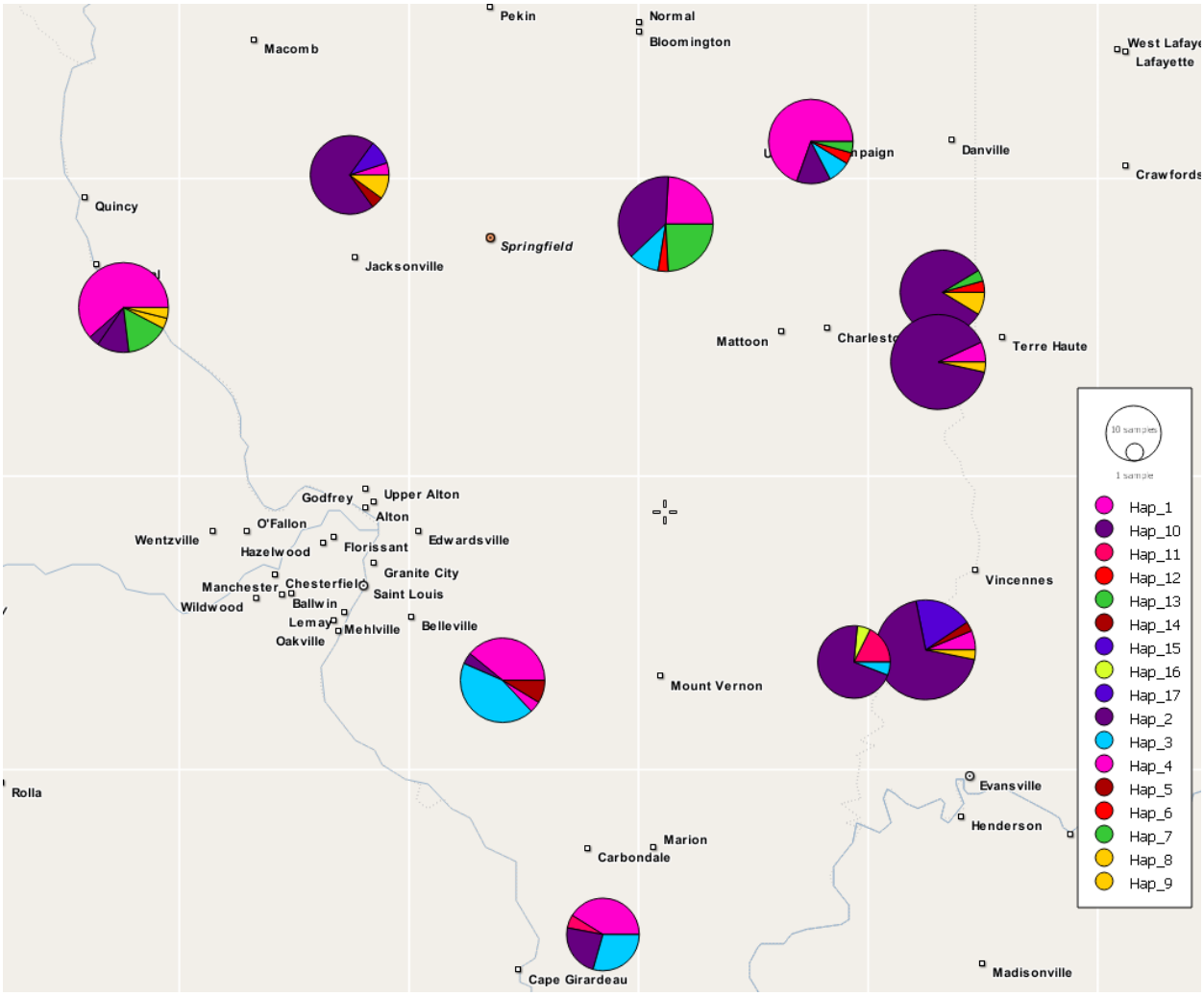

Fig 7: A map depicting the proportions of each location's samples belonging to the different haplotypes.

Discussion

Of the 24 species collected from 8 communities in 2017 using BG-Sentinel traps, 4 mosquito species were found in all sampling sites: *Ae. albopictus*, *An. quadrimaculatus*, *Cx. erraticus* and *Cx. pipiens*. For comparison, we detected 22 mosquito species in 10 larger communities along the I-57 in 2016 and 5 mosquito species were common to all those sites: *Ae. albopictus*, *Ae. canadensis*, *Anopheles crucians*, *An. punctipennis* and *Psorophora columbiae*. Considering all 18 communities surveyed together, only *Ae. albopictus* was consistently detected from all locations. The difference of the number of species between 2016 and 2017 surveys is most likely due to additional molecular species identification included for the 2017 survey, while only morphological species identification (resulting in a greater number of genus-level only identifications) was performed for the 2016 survey.

Anecdotally, the communities surveyed in 2016 were larger and more urbanized than the communities surveyed in 2017. These are rich and detailed data sets that will allow us to investigate the environmental determinants of mosquito community structure in more detail in the future. We are particularly interested in investigating whether land cover and environmental variables can explain differences in the abundance of *Ae. albopictus*, which showed considerable variation among sites. This would not only help us explain why certain areas have greater infestations, but possibly aid in predicting which areas in Illinois may be prone to future *Ae. albopictus* population expansion.

The survey for adult mosquitoes revealed that *Ae. albopictus* was ubiquitously present in all communities in the current study, but *Ae. aegypti* was not detected in any of the surveyed locations. The latter is not entirely surprising, as (the southern part of) Illinois only just falls in the "extreme range" or "temporary summer region" of this species (Darsie & Ward 2005; Eisen & Moore 2013). Collections from these northernmost parts of the range of *Ae. aegypti* are typically low in number and most likely represent new and seasonal introductions, likely resulting from tire shipments, during the summer. It is notable though that this species appears to be reinvading areas in which it had been presumed absent for years (Zohdy *et al* 2018); and examples such as the occurrence of this species as far north as Windsor, Canada, suggests the possibility of establishment and expansion during the summer in Illinois should not be discounted entirely (Monaghan *et al*. 2016).

The counties where *Ae. albopictus* was detected are Vermilion, Edgar, Clark, Crawford, Lawrence, Wabash, Edwards and Wayne, represented by cities of Danville, Paris, Marshall, Robinson, Lawrenceville, Mt. Carmel, Albion and Fairfield in Illinois. These findings could suggest that *Ae. albopictus* is continuing to expand its distribution in Illinois, or alternatively that the previous absence of occurrence records in these counties was due to a lack of surveillance (or use of traps that are less likely to pick up this species). Although there remain gaps in our knowledge, we infer that this species is now established throughout the southern half of the state. Questions that remain are: how far north can we find populations and where does the boundary between established (and overwintering) versus seasonal populations lie? In Champaign, the abundance of *Ae. albopictus* has drastically increased over the past three years (J. Blackford, A. Parker, personal communication). An important question is whether such increases have been consistent across the southern half of Illinois; and if not, what are reasons for this variation? Given the results of the mitochondrial DNA sequencing, it is clear that distinct

genetic lineages are present in Illinois. Several important questions arise from this initial investigation: to what extent do these different haplotypes differ phenotypically? For instance, are there differences in their cold tolerance and ability to overwinter (which could lead to further expansion, or a more rapid increase in population numbers during early summer)? Or, are there behavioral differences between these populations? It would also be useful to unravel the invasion history of these different haplotypes to gain insight into whether an initial population is being displaced by one that is potentially more well-adapted to a northern climate. Future experimental and surveillance work following up on this, making use of molecular markers along a wider range of the genome, would be required to elucidate these questions.

As *Ae. albopictus* is a highly competent vector for a wide range of arboviruses, the risk of arboviral outbreaks continues to pose a threat. While for viruses such as dengue or Zika, a local outbreak in Illinois would require a confluence of events, WNV is ubiquitous in Illinois and *Ae. albopictus* is, at least in the lab, a competent vector (Sardelis et al. 2002). It may also serve as a bridge vector for EEE and La Crosse (Gratz 2004). Understanding how the expansion and increase of *Ae. albopictus* populations changes the landscape of arboviral transmission risk across Illinois will thus have to be an active area of research going forward.

Besides *Ae. albopictus*, the survey results indicated that *Ae. vexans*, *Cx. erraticus* and *Cx. pipiens* were ubiquitously present in the surveyed communities. Although typically thought of as a nuisance biting mosquito, at least one study has shown that field-collected *Ae. vexans* are capable of transmitting Zika virus (O'Donnell et al. 2017), and may also serve, in certain situations, as a bridge vector of WNV (Kilpatrick et al. 2005). As these species (and others, such as *Ae. japonicus* and *Ae. triseriatus*) can interact to an extent with *Ae. albopictus*, it will be important to understand whether their role in transmission of WNV, LACV, or other arboviruses, is altered as a result of their ecological interactions. All collected mosquito specimens were tested, but no mosquito pool produced positive results for WNV, ZIKV, JCV, EEEV, LACV and DENV. Although the findings seem encouraging from a public health perspective, the numbers of mosquitoes collected from the BG traps were still relatively low. Arboviral infection rates in mosquitoes are often well below 1%, even in samples collected from gravid traps. In other words, the odds of finding a positive mosquito in these samples, even if viruses were circulating in these locations, was probably quite low. Dedicated surveillance efforts aimed at obtaining sufficient sample sizes (i.e., trapping more frequently and with a greater number of traps in a given location), or an alternative virus surveillance method (e.g, a sugar-feeding based surveillance system [van den Hurk *et al.*, 2014]) would be necessary to obtain better insight into the actual role of *Ae. albopictus*, both through its ecological impact on other mosquito species and in its capacity as a vector itself, on arboviral transmission in Illinois.

Acknowledgements

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Table 1. List of communities and sampling locations in Southern/Central Illinois

City	Site No.	GPS Coordinates	Address
Danville	1	40.193390, -87.729201	22296 Henning Road, Danville, IL 61834
	2	40.193623, -87.729241	22296 Henning Road, Danville, IL 61834
	3	40.193792, -87.729372	22296 Henning Road, Danville, IL 61834
Paris	4	39.619235, -87.665956	Clinton Ave., Paris, IL 61944
	5	39.632737, -87.676862	10998 N. 1545th St., Paris, IL 61944
	6	39.603167, -87.692908	210 E. Jasper St., Paris, IL 61944
Marshall	7	39.393856, -87.697275	601 W. Hickory St., Marshall, IL 62441
	8	39.385059, -87.694908	707 S. 5th St., Marshall, IL 62441
	9	39.389315, -87.681043	1670 E. Plant Rd., Marshall, IL 62441
Robinson	10	39.014189, -87.724696	1000 N. Mitchell Ave, Robinson, IL 62454
	11	39.014746, -87.724735	1000 N. Mitchell Ave, Robinson, IL 62454
	12	39.014293, -87.726260	1000 N. Mitchell Ave, Robinson, IL 62454
Lawrenceville	13	38.732442, -87.690197	18th St. Cemetery, Lawrenceville, IL 62439
	14	38.732185, -87.689776	18th St. Cemetery, Lawrenceville, IL 62439
	15	38.732071, -87.689063	18th St. Cemetery, Lawrenceville, IL 62439
Mt. Carmel	16	38.409627, -87.749492	Golden Aces Way, Mt Carmel, IL 62863
	17	38.399562, -87.771948	112 Division St. Mt Carmel, IL 62863
	18	38.400728, -87.771835	112 Division St. Mt Carmel, IL 62863
Albion	19	38.368024, -88.062532	S. 8th, Albion, IL 62806
	20	38.367996, -88.061788	S. 8th, Albion, IL 62806
	21	38.368590, -88.062965	S. 8th, Albion, IL 62806
Fairfield	22	38.370029, -88.340335	798 County Highway 6, Fairfield, IL
	23	38.370149, -88.340615	798 County Highway 6, Fairfield, IL
	24	38.368962, -88.338346	798 County Highway 6, Fairfield, IL

Table 2. Mosquito species and numbers collected by BG-Sentinel traps in 8 communities of Southern/Central Illinois.

	Danville	Paris	Marshall	Robinson	Lawrenceville	Mt. Carmel	Albion	Fairfield
Aedes albopictus	1	228	79	6	2	140	21	4
Aedes canadensis	397	0	0	191	86	171	5	5
Aedes japonicus	0	1	2	0	0	0	0	0
Aedes spp	1	0	0	1	0	3	0	0
Aedes triseriatus	3	1	0	0	0	6	0	0
Aedes trivittatus	8	0	26	0	0	23	0	0
Aedes vexans	92	18	88	36	33	75	11	5
Anopheles crucians	0	0	0	0	0	0	1	0
Anopheles punctipennis	8	0	4	16	1	1	4	3
Anopheles quadrimaculatus	7	3	7	7	3	6	1	1
Anopheles spp	0	1	0	0	8	4	0	0
Culex erraticus	24	8	11	14	65	48	3	1
Culex nigripalpus	0	0	4	0	0	0	0	0
Culex pipiens	19	29	70	34	56	217	74	16
Culex restuans	0	12	7	1	0	4	41	3
Culex salinarius	2	1	1	8	0	10	0	0
Culex spp	3	4	1	0	1	7	1	1
Culex territans	1	0	0	2	1	0	0	0
Orthopodomyia signifera	0	1	0	0	0	0	0	0
Psorophora ciliata	0	0	2	0	0	0	0	0
Psorophora columbiae	0	0	9	0	0	20	1	0
Psorophora confinnis	0	0	0	0	0	1	0	0
Psorophora ferox	0	0	0	0	0	3	1	0
Psorophora horrida	0	0	0	0	0	1	0	0
Psorophora howardii	0	0	0	0	1	0	0	0
Toxorhynchites spp	0	0	0	1	0	0	0	0
Uranotaenia sapphirina	0	0	0	0	2	0	0	0

Table 3. Mosquito species and numbers collected by oviposition traps in 8 communities of Southern/Central Illinois

	Danville	Paris	Marshall	Robinson	Lawrenceville	Mt. Carmel	Albion	Fairfield
Aedes albopictus	0	104	126	56	27	118	74	29
Aedes triseriatus	0	0	11	1	5	1	7	0
Aedes vexans	1	0	0	0	0	0	0	0
Anopheles crucians	0	0	0	2	0	1	0	0
Anopheles spp	0	0	2	1	0	5	22	9
Anopheles walkeri	0	0	0	0	0	0	0	1
Coquillettidia perturbans	0	2	1	0	0	0	0	0
Culex pipiens	7	2	17	17	11	9	50	26
Culex restuans	0	1	2	2	0	0	2	0
Culex spp	0	2	5	14	1	10	0	1
Culex territans	0	0	0	0	2	0	0	0
Orthopodomyia signifera	0	0	1	0	0	0	12	0

Table 4. Mosquito species and numbers collected by gravid traps in 8 counties of Illinois

	Adams	Jackson	Kane	Macon*	McLean	Sangamon	St. Clair	Will**
Aedes albopictus	63	27	0	637	16	78	33	N/A
Aedes canadensis	2	0	0	N/A	0	0	0	N/A
Aedes grossbecki	1	0	0	N/A	0	0	0	N/A
Aedes japonicus	23	38	23	N/A	27	4	203	N/A
Aedes spp	0	2	3	N/A	0	7	2	N/A
Aedes triseriatus	13	2	0	N/A	1	0	3	N/A
Aedes trivittatus	1	0	1	N/A	0	0	0	N/A
Aedes vexans	4	0	0	N/A	0	0	0	N/A
Anopheles crucians	0	6	0	N/A	0	4	0	N/A
Anopheles punctipennis	0	12	0	N/A	0	2	1	N/A
Anopheles quadrimaculatus	2	0	0	N/A	0	3	0	N/A
Anopheles spp	0	8	1	N/A	0	22	2	N/A
Culex pipiens	0	2	0	N/A	0	33	1	N/A
Culex restuans	0	5	0	N/A	0	1	0	N/A
Culex spp	0	6	0	N/A	0	8	1	N/A
Culex tarsalis	0	0	0	N/A	0	0	1	N/A
Orthopodomyia signifera	1	0	0	N/A	0	0	0	N/A

* Mosquito species identification was performed by Macon County mosquito abatement district and only *Ae. albopictus* specimens were sent to INHS.

**No mosquito was found from the specimens from Will County.

Table 5. Mosquito species diversity and evenness for communities surveyed by BG-Sentinel traps. The values in parentheses represent the number of mosquito species collected in the survey.

City	Shannon Index	Equitability
Danville	1.12 (9)	0.45 (9)
Paris	1.02 (9)	0.44 (9)
Marshall	1.93 (11)	0.75 (11)
Robinson	1.48 (10)	0.58 (10)
Lawrenceville	1.79 (8)	0.72 (8)
Mt. Carmel	1.93 (11)	0.75 (11)
Albion	1.59 (10)	0.66 (10)
Fairfield	2.06 (8)	0.86 (8)