Zika vector competence data reveals risks of outbreaks: the contribution of the European ZIKAlliance project

First identified in 1947, Zika virus took roughly 70 years to cause a pandemic unusually associated with virus-induced brain damage in newborns. Zika virus is transmitted by mosquitoes, mainly *Aedes aegypti*, and secondarily, *Aedes albopictus*, both colonizing a large strip encompassing tropical and temperate regions. As part of the international project ZIKAlliance initiated in 2016, 50 mosquito populations from six species collected in 12 countries were experimentally infected with different Zika viruses. Here, we show that *Ae. aegypti* is mainly responsible for Zika virus transmission having the highest susceptibility to viral infections. Other species play a secondary role in transmission while *Culex* mosquitoes are largely non-susceptible. Zika strain is expected to significantly modulate transmission efficiency with African strains being more likely to cause an outbreak. As the distribution of *Ae. aegypti* will doubtless expand with climate change and without new marketed vaccines, all the ingredients are in place to relive a new pandemic of Zika.
Viral pathogens with high epidemic potential have marked human history by massive losses of life and economic declines. Pandemics like the Spanish flu have left traces and the fear of new viral emergences materializes today with the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic. Over the past decades, efforts invested into vaccination programs and development of antiviral treatments have led to major medical progress, resulting in measles vaccination, smallpox eradication, appropriate treatments of Human Immunodeficiency virus and Hepatitis C virus, and improved control of SARS-CoV-2. As major viral pathogens, arthropod-borne viruses (arboviruses) are also on the rise. Arboviruses have developed a strategy to accomplish their transmission cycle via replication in both vertebrate and arthropod hosts. Their RNA genome contributes to the generation of large populations of genetically distinct variants permitting adaptations to environmental changes including host switching and transmission. In the last 30 years, mosquito-borne viruses have dramatically expanded their distribution range leading to increasingly frequent and large epidemics. In 2016, Zika was designated as a “Public Health Emergency of International Concern” by the World Health Organization, subsequently to the large spread of Zika virus (ZIKV) in the Pacific islands and the Americas with unusual notifications of microcephaly in newborns and different neurological disorders (e.g. Guillaum-Barre syndrome); this happened 70 years after its first isolation from a human case in East Africa. Local cases of Zika were detected in 2019 in Southern France additionally corroborating the role of the Asian tiger mosquito (Aedes albopictus) in ZIKV transmission in addition to chikungunya and dengue viruses.

Aedes mosquitoes are important vectors of arboviruses, acquiring the virus through a blood meal from an infected host. Subsequently, the virus replicates within the mosquito and is transmitted to a new host. The virus must overcome two anatomical barriers (midgut and salivary glands) and circumvent major antiviral pathways before being delivered in saliva. Geographic populations of a same mosquito species may not share the same immunological background, leading to different vector competences. The outcome of infection depends on specific pairings of mosquito and pathogen genotypes described under genotype-by-genotype (G x G) interactions. These interactions could structure viral populations through adaptations to their local vector populations. Vector competence data have been used to derive risk maps informed with outcomes of infection depends on specific pairings of mosquito and pathogen genotypes described under genotype-by-genotype (G x G) interactions. These interactions could structure viral populations through adaptations to their local vector populations. Vector competence data have been used to derive risk maps informed with outcomes of infection. For all three outcomes, for the African ZIKV strain, a regular decrease was observed between IR (73.9%), DE (69.2%) and TE (55.8%), consistent with a lack of viral progression through the midgut, and partial barriers to viral progression before reaching the salivary glands. In contrast, when infected with Asian ZIKV strains, both midguts and salivary glands mitigated progression of the virus: IR = 68.9% [64.3%–73.3%], DE = 56.7% [51.8%–61.5%], and TE = 28.9% [24.3%–33.5%].

In Aedes albopictus mosquitoes, viral progression was more efficient with the African ZIKV strain than with American or Asian ZIKV strains for all three outcomes. For the African ZIKV strain, a regular decrease was observed between IR (73.9%), DE (69.2%) and TE (55.8%), consistent with the absence of a marked barrier to viral progression through the midgut and into the salivary glands. In contrast, DE was significantly lower for American (15.6% [11.5%–20.4%]) and Asian (11.2% [7.7–15.5%]) ZIKV strains, consistent with a lack of viral progression through the midgut.

Time since infection showed an increasing, approximately linear association with DE and TE in Aedes aegypti and Aedes albopictus mosquitoes, suggesting an accumulation pattern and an overall absence of viral clearance within the vector, while IR reached a peak value within 7 dpi and remained at comparable levels until 21 dpi. In contrast, Aedes japonicus infected by the African ZIKV strain showed that IR peaked at 14 dpi, with decreasing DE and TE values over time (TE_{14dpi} = 15.0% [7.1–26.6%], TE_{26dpi} = 15.0% [7.1–26.6%] and TE_{26dpi} = 6.7% [18.5–16.2%]).

Transmission efficiency is higher with the African ZIKV. Although all species of Aedes studied were competent to transmit ZIKV, infected Aedes aegypti mosquitoes were on average more prone to successful dissemination than Aedes albopictus and Aedes japonicus (TE_{aegypti} = 34.0% [24.0–45.8%], TE_{albopictus} = 17.6% [11.4–26.0%], TE_{japonicus} = 7.2% [2.9–16.9%]). This was even more pronounced at 21 dpi when TE was significantly higher for Aedes aegypti than Aedes albopictus (46.7% [35.3–58.5%] vs. 25.3% [17.3–35.3%, p < 0.0001] and Aedes japonicus (46.7% [35.3–58.5%] vs. 10.8% [4.4–24.1%, p = 0.0002]), the latter two not reaching statistical significance (p = 0.09). However, many combinations of ZIKV strains and mosquito populations were not available for Aedes japonicus for which results should be considered less robust.

The African ZIKV strain was the most efficient in infecting and disseminating, therefore posing a greater risk in case it was newly introduced in a region: the predicted expected marginal means 21 dpi were TE = 82.7% [78.5–86.1%] for Aedes aegypti and TE = 52.4% [45.4–59.3%] for Aedes albopictus (Fig. 2). American and Asian strains had comparable TE regardless of time since infection: (I) in Cameroon,
Congo, and Gabon, TE was low (~10%), suggesting African mosquitoes are mostly susceptible to local ZIKV strains; (2) in Brazil, Haiti, Guadeloupe, Madeira, La Reunion and New Caledonia, TE reached moderate values ~20%. In Cambodia however, Asian and American ZIKV strains had much higher TE values, denoting mosquito populations highly susceptible to ZIKV.

Breaking down variable importance in ZIKV susceptibility

We evaluated the extent to which each covariate of interest contributed to explanatory value of fitted models. For both *Ae. aegypti* and *Ae. albopictus*, TE showed low variability among mosquito populations originating from the same country (Fig. 3). Another striking result from Fig. 3 confirmed the higher susceptibility to African ZIKV strains among all tested mosquitoes, both within and across countries. This suggests that within a given country, mosquito populations were equally able to disseminate ZIKV regardless of other extrinsic factors. We found that 51.7% of variance in *Ae. aegypti* TE could be explained by a statistical model accounting for mosquito strain, ZIKV strain, and dpi (Table 1). The relative importance of these factors was assessed by estimating the proportion of variance explained solely by these factors. For *Ae. aegypti* TE, ZIKV strain was the most important factor explaining 33.5% of variance, followed by dpi (30.1%). Of the factors considered, mosquito strain had the least impact on TE, with only 6.6% of variance explained by the country of a sampled mosquito strain. An additional 2.6% of variance was explainable when we considered locations of strains within a country. For *Ae. japonicus*, no obvious pattern could be detected with only 5.2% of the observed variance explained by available covariates suggesting that mosquitoes were less susceptible to tested ZIKV strains.

Discussion

Our results confirmed that human-adapted *Aedes* mosquitoes prepare the field for new arbovirus emergences; in addition to other factors, some combinations of vector-virus are more prone to cause outbreaks, but globalization will smooth out differences leading most emergences to turn into pandemics owing to the large territories covered by vectors.

During the 2015–2016 Latin American and the Caribbean epidemic, the role of *Culex* as ZIKV vector has been the subject of major scientific debates which, if verified, would have revised the *modus operandi* of vector control. As ZIKV has also been detected in field-collected *Culex* mosquitoes, their susceptibility to ZIKV was then tested; from 36 studies on vector competence of *Culex* mosquitoes for ZIKV, seven studies showed that ZIKV was present in the saliva of *Cx. restuans*, *Cx. quinquefasciatus*, *Cx. tarsalis*, and *Cx. Coronator*. Asian and American genotypes of ZIKV may occasionally infect *Culex* mosquitoes but not African strains. From our dataset, we showed that ZIKV can accumulate in *Cx. pipiens* saliva only after forced infection by intrathoracic inoculation, short-circuiting the midgut barrier; after oral infection, the virus was unlikely to disseminate in the mosquito ruling out the role of *Culex* mosquitoes in ZIKV.
transmission. Viral replication was not detected in *Culex* which was unable to trigger the RNAi pathway as no viral replication signatures such as 21 nt viral siRNAs were detected19. Eventhough vectors of the flavivirus West-Nile virus, a phylogenetically ZIKV-related arbovirus, mosquitoes of the *Cx. pipiens* complex are not competent for ZIKV transmission and are very unlikely to play a role of vector during a Zika outbreak. Thus, to control Zika, targeted interventions should focus largely on *Aedes* mosquitoes11,20. *Aedes aegypti* is an African mosquito (named *Ae. aegypti formosus*) living primarily in rainforests colonizing natural breeding sites which in a subsequent step, has become adapted to living in the human environment (developing a human-biting behavior and colonizing human-made containers) before undergoing different waves of dissemination outside Africa21. The “domestication” of this species (named *Ae. aegypti aegypti*) allowed its world-wide dispersion coinciding with the first twentieth century pandemics caused by arboviruses, dengue22, and then chikungunya23 and Zika24. In addition to increased vector-host contacts, *Ae. aegypti aegypti* has developed an enhanced permissiveness to some arboviruses7. At the mosquito level, successful transmission implies that the virus passes through two anatomical barriers, midgut and salivary glands25. *Ae. aegypti* mosquitoes we tested were more prone to impose a strong bottleneck to the African ZIKV in the salivary glands leading to decrease ZIKV transmission. Nevertheless, all 11 *Ae. aegypti* populations transmitted at least 2–3 times more the African ZIKV than the American and Asian ZIKV strains underlining the high potential of African ZIKV to trigger an outbreak outside and inside.
Africa. It is now admitted that African ZIKV strains were more pathogenic than the Asian strains. African Ae. aegypti (Cameroon, Congo, Gabon) were more resistant to American and Asian ZIKV than to African ZIKV suggesting a local adaptation between a ZIKV strain and a mosquito population. There is evidence today that Ae. aegypti formosus is found both in urban and forest habitats in sub-Saharan Africa and a mixture of Ae. aegypti aegypti and Ae. aegypti formosus is likely present in cities we sampled in Cameroon, Congo and Gabon.

As Ae. aegypti aegypti is more susceptible to ZIKV than Ae. aegypti formosus, an intermediate level of susceptibility can be expected. Ae. aegypti aegypti, which is predominant in Benoué, was previously found less susceptible to ZIKV compared to the other populations from Cameroon. Nevertheless, mosquitoes were only identified according to morphological criteria and not based on their genotypes. As such, the observed local reduction in vector competence may also result from other inherited factors not detected through morphological examinations. Our experiments showed a decreased susceptibility within the country, with Ae. aegypti from Benoué having lower TE (34.4% [20.2–52.1%]) than those sampled elsewhere in Cameroon (ranging from 62.5 to 83.3%, Supplementary Table 2) except in Maroua, located close to Benoué. Intriguingly, human epidermics of ZIKV associated with Ae. aegypti have been observed in a limited number of countries in Asia or Africa following the 2015–2016 Latin American and the Caribbean epidemic. The Asian Ae. aegypti population from Cambodia we tested, transmitted the most efficiently all three ZIKV strains, African, American and Asian, corroborating its good vector competence to ZIKV. Recent systematic screenings of patients revealed that ZIKV was circulating in Singapore, Thailand, Cambodia, Myanmar and Vietnam suggesting that ZIKV has been present in Asia at a low but sustained level for years or even decades. Moreover, human population immunity could also modulate the risk of outbreaks; a large population exposed to dengue viruses may develop neutralizing antibodies against dengue viruses which cross-react with ZIKV adjusting the outcome of ZIKV infection. Thereby, Asian populations can be partially protected against ZIKV. At an intra-country level, mosquito populations (in our study, e.g. five in Brazil) share the same profiles of transmission with a highest transmission level with African ZIKV. Once introduced, the African ZIKV has the potential to cause an outbreak whatever the origin of Ae. aegypti populations. Our assessments are based on vector competence which is only one aspect of vectorial capacity; it results from complex interactions of multiple factors influencing pathogen transmission by a vector, including internal factors such as genetic, evolution, immunity, or interactions with other microorganisms, modulated by external factors such as abiotic (like climate or topography) and biotic (like nutrition or hosts).

In the tropical belt, Ae. aegypti and Ae. albopictus share the same ecological niche. Ae. albopictus mosquitoes we tested were more susceptible to African ZIKV suggesting that the species can contribute to an epidemic like it happened in Gabon in 2007. A lower transmission of American and Asian ZIKV was presumably related to a strong mosquito midgut barrier. Since its arrival in Central Africa in 2000, Ae. albopictus plays a major role in the transmission of different arboviruses. Ae. albopictus tends to predominate over Ae. aegypti in habitats where both species are sympatric and will certainly promote arbovirus spillovers at the edge of their natural sylvatic cycle owing to its generalist behavior (biting both humans and animals, and living in rural areas). Ae. albopictus from Central Africa are likely originated from South America which themselves were introduced from North America in 1986. European Ae. albopictus are presumably a genetic mixture of populations from United States and La Reunion, sharing similar profiles of ZIKV transmission with mosquitoes from La Reunion. American ZIKV predominant during the 2015–2016 epidemic, was efficiently transmitted by Ae. albopictus, in Europe and Brazil. In the absence of Ae. aegypti, Ae. albopictus is more likely to trigger ZIKV outbreaks when ZIKV comes from Africa.

Another invasive species, Ae. japonicus also experimentally transmits the African ZIKV. Native to East Asia, Ae. japonicus arrived in Europe in 2000 and is established in 12 European countries up to now. Its role in pathogen transmission is still unclear even if arboviral ZIKV RNAs have been detected in field-collected mosquitoes. Its high ability to become infected, and to a lower extent, to ensure viral dissemination and transmission, concomitantly to its human-biting behavior gives Ae. japonicus the status of being a competent vector of ZIKV. These findings are however subject to caution, as the experimental design may not allow for generalization of our observations: Ae. japonicus could only be sampled and bred in European countries (France (n = 90), Switzerland (n = 90), the Netherlands (n = 62)). To add to this limitation, only two ZIKV strains were tested against these mosquitoes and fully collinear with the country of mosquito sampling: French- and Swiss-sampled Ae. japonicus were infected with an African ZIKV strain, whereas mosquitoes from the Netherlands were infected with an American ZIKV strain (see data structure in Supplementary Fig. 1). Therefore, we cannot distinguish between mosquito susceptibility and ZIKV strain potency.

We conclude that from our comprehensive assessment of vector competence, invasive Aedes mosquitoes set the bed for pandemic arboviruses; in the absence of the historical vector of human arboviruses, Ae. aegypti, other species such as Ae. albopictus or Ae. japonicus can take over as ZIKV vectors and transmit even more efficiently when infected with the African ZIKV.

### Methods

#### Experimental procedures

All partners of the ZIKAlliance project (https://zikalliance.tghn.org/) performed their own mosquito experimental infections using a common standardized protocol (Supplementary Fig. 2). Some deviations from this protocol could not be avoided (e.g. availability of blood source, feeding via blood droplets instead of via membrane). Fifty populations (Fig. 4 and Supplementary Table 1) were collected as immature stages and reared in insectaries under controlled conditions. Mosquito females were exposed to an infectious blood meal containing one third of viral suspension and two third of washed rabbit erythrocytes (Supplementary Fig. 3) at a final titer of -10 TCID_{50}/ml. After 1 h, engorged females were isolated in boxes (28±1°C and 80±10% humidity) and fed with 10% sucrose until analysis.

### Table 1 | Contribution of random effects and fixed effects in species-wise regression models

| Mosquito species | Total variance explained | Variance uniquely explained |
|------------------|-------------------------|-----------------------------|
|                  | Within-country (mosquito) | Between-country (mosquito) | ZIKV strain | Days post-infection |
| Ae. aegypti      | 51.7%                    | 2.6%                        | 6.5%        | 33.5%               | 30.1%               |
| Ae. japonicus    | 5.2%                     | 1.0%                        | 0.0%        | 0.0%                | 1.0%                |
| Ae. albopictus   | 41.5%                    | 1.7%                        | 0%          | 29.0%               | 21.2%               |

The contribution of random effects was measured as the adjusted intra-class correlation coefficient (as defined in Nakagawa et al.), while the contribution of fixed effects was assessed by the semi-partial R² measure (from Stoffel et al.), i.e. the share of variance uniquely explained by a given covariate. The total variance explained is the R² measure of the saturated model.
Different geographic ZIKV strains were used (Supplementary Fig. 4): (1) Africa (Dakar; KU955592; isolated in 1984 from *Aedes taylori* in Senegal and passaged four times on BHK21 cells), (2) Asia (Cambodia; KU955593; isolated from a human case in 2010 and passaged three times on Vero cells and Malaysia; KX694533; isolated in 1966 from *Aedes aegypti* in Malaysia and passaged four times on Vero cells), and (3) America (Guadeloupe; LR792671.1; isolated from a human case in Guadeloupe in 2016 and passaged two times on Vero cells, Martinique; KU647676; isolated from a human case in 2015 and passaged three times on Vero cells, and Suriname; KU937936; isolated from a human serum in Suriname in 2016 and passaged 5–6 times on Vero cells). ZIKV Dakar, Malaysia, and Martinique provided by EVAg (https://www.european-virus-archive.com/) were used in most infections. ZIKV Cambodia was used for the mosquito populations Haiti, Funchal, Mont, Corse, ZIKV Suriname for Best, Amsterdam and Lelystad, and ZIKV Guadeloupe for HAV-PT, HAV-PRG.

At 7, 14, and 21 days post-infection (dpi), a batch of mosquitoes was processed to estimate if viral particles were present in the body (thorax and abdomen) as the marker for infection, head for dissemination and saliva for transmission; 0 refers to no viral particles detected and 1 to at least one viral particle detected. Presence of viral particles was asserted by cytopathic effects observed on a monolayer of Vero CCL-81 cells. IR was asserted by cytopathic effects observed on a monolayer of Vero cells, and 1 to at least one viral particle detected. Presence of viral particles is a marker for infection, head for dissemination, and saliva for transmission; 0 refers to no viral particles detected and 1 to at least one viral particle detected. Progression of infections was measured as the adjusted intra-class correlation coefficient (as defined by Nakagawa et al.52). Likewise, the contribution of random effects was measured as the adjusted intra-class correlation coefficient (as defined by Nakagawa et al.52). Analyses were conducted using the R software (v. 4.2.0)16 with the following packages: lme4 (v. 1.1-27.1)54, emmeans (v. 1.7.2), performance (v.0.8.0)55 and partR2 (v. 0.9.1).

The robustness of results was assessed using sensitivity analyses, where the same regression models were applied with data aggregated at larger geographical scales for covariates of interest. Three variations of the same analyses were conducted: (1) by aggregating country of study by continent (therefore assuming random variations across countries) and preserving individual ZIKV strains, (2) by aggregating ZIKV strains by continent (corresponding to relatedness between strains in the phylogenetic tree, see Supplementary Fig. 4) and preserving individual countries of study, and (3) aggregating both country and virus strain by continent. The results from aggregation detailed in point (2) are presented in the main text as they provide the most comprehensive set of results. The results corresponding to aggregation levels from points (1), (3) and (4) are presented in Supplementary Figs. 5 through 10.

**Statistical analysis**

Success for transmission, dissemination and infection were investigated as binomial outcomes to assess how their population-level counterpart (TE, DE, IR) were associated with available combinations of mosquito populations and ZIKV strains, according to time since infection. Time since infection was considered a categorical covariate. Prior to any modeling approach, all outcomes were visualized with heat maps similar to those presented in Supplementary Figs. 6, 8 and 9, for all combinations of ZIKV strain and mosquito population and at every value of time since infection. The resulting experimental design, consisting of combinations of country of study, mosquito species and populations as well as time since infection, was used to guide the development of generalized linear (mixed) models.

Owing to complex hierarchical experimental design, each mosquito species was modeled separately. When several mosquito populations were sampled at different locations within a given country, the variations resulting from the population effect was modeled by a random effect nested within the country, in a mixed regression framework. The contribution of each fixed-effect term was assessed by refitting univariable models and measuring the semi-partial R-squared value (as defined by Stoffel et al.57). Likewise, the contribution of random effects was measured as the adjusted intra-class correlation coefficient (as defined by Nakagawa et al.52). Analyses were conducted using the R software (v. 4.2.0)16 with the following packages: lme4 (v. 1.1-27.1)54, emmeans (v. 1.7.2), performance (v.0.8.0)55 and partR2 (v. 0.9.1).

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In the article, the authors discuss the role of two populations of Aedes mosquitoes in the transmission of Zika virus. They conducted an experiment where they exposed Aedes albopictus mosquitoes to a fluctuating temperature regime and observed the transmission of the virus. The results showed that the virus was transmitted efficiently under these conditions.

The authors also mention that the Asian bush mosquito Aedes japonicus can experimentally transmit Zika virus to humans and that Usutu virus was detected in saliva of the progeny. They conclude that the transmission of Zika virus is influenced by various factors such as temperature, mosquito species, and demographic history.

The competing interests section states that the authors declare no competing interests. They also note that the study was supported by the European Union’s Horizon 2020 research and innovation program under ZIKAlliance grant agreement no. 734548.

The Acknowledgements section thanks Sally Paulson for her contribution to the peer review of this work. They also acknowledge the help of the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Additional information includes supplementary material available at https://doi.org/10.1038/s41467-022-32234-y.

Peer review information notes that Nature Communications thanks Sally Paulson and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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