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Gone (and spread) with the birds: Can chorotype analysis highlight the spread of West Nile virus within the Afro-Palaearctic flyway?

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ABSTRACT

West Nile virus (WNV) is a globally significant vector-borne disease that is primarily transmitted between birds and mosquitoes. Recently, there has been an increase in WNV in southern Europe, with new cases reported in more northern regions. Bird migration plays a crucial role in the introduction of WNV in distant areas. To better understand and address this complex issue, we adopted a One Health approach, integrating clinical, zoological, and ecological data. We analyzed the role of migratory birds in the Palaearctic-African region in the spread of WNV across Africa and Europe. We categorized bird species into breeding and wintering chorotypes based on their distribution during the breeding season in the Western Palaearctic and the wintering season in the Afrotropical region, respectively. By linking these chorotypes to the occurrence of WNV outbreaks in both continents throughout the annual bird migration cycle, we investigated the relationship between migratory patterns and virus spread. We demonstrate that WNV-risk areas are interconnected through the migration of birds. We identified a total of 61 species that potentially contribute to the intercontinental spread of the virus or its variants, as well as pinpointed high-risk areas for future outbreaks. This interdisciplinary approach, which considers the interconnectedness of animals, humans, and ecosystems, represents a pioneering effort to establish connections between zoonotic diseases across continents. The findings of our study can aid in anticipating the arrival of new WNV strains and predicting the occurrence of other re-emerging diseases. By incorporating various disciplines, we can enhance our understanding of these complex dynamics and provide valuable insights for proactive and comprehensive disease management strategies.

1. Introduction

West Nile virus (WNV) is a single-stranded RNA virus (genus *Flavivirus*) that causes West Nile fever. It is transmitted through mosquitoes (vectors), particularly the *Culex* species, and is amplified in birds, which serve as reservoirs for the virus. While humans and horses can also become infected, they are considered dead-end hosts Spillover from the enzootic cycle occasionally results in serious outbreaks among, both [1]. Most infected humans remain asymptomatic, but around 20% develop influenza-like symptoms. In a few cases (<1%), severe neurological symptoms can lead to death [2].

The discovery of WNV dates back to 1937 when it was first isolated in Uganda [3]. By 1950, outbreaks of the disease were documented in North Africa [4,5]. The presence of high levels of WNV antibodies in human across Africa suggests that the virus may be endemic in numerous regions of the continent [5,6]. However, the actual disease burden in the continent is likely underestimated [6,7]. In Europe, the first outbreak of WNV was reported in 1962 in the Camargue region of France [8]. The first significant outbreak occurred in 1996 in Eastern Europe [9]. Since then, outbreaks have been regularly reported in Europe, with a marked seasonal pattern from July to October [10].

The role of Palaearctic-African migratory birds in introducing WNV

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to Europe has long been suspected. The virus is believed to circulate more prominently during late summer when migratory birds and mosquitoes coexist in large concentrations of [4,11]. Phylogenetic analyses of the virus responsible for a major outbreak in Romania in 1996 supported the hypothesis of WNV transfer from sub-Saharan Africa to Europe by migratory birds [12]. Furthermore, the detection of WNV RNA in mosquitoes during winter in 2017, provided evidence of overwintering of the virus in Europe, independent of new introductions of the virus by migratory birds [13]. Initially, only WNV 1 strains were known to circulate in Europe, but in 2004, lineage 2, previously found in sub-Saharan Africa and Madagascar (**Supplementary Fig. 1**), was isolated from a goshawk (*Accipiter gentilis*) in Hungary [14]. Currently, most human cases in Europe are caused by lineage 2 [15], and multiple introductions of lineage 2 occurred in the continent [16–18]. There is also evidence that the lineage 1 has experienced several independent introductions into Europe [19]. The appearance of new strains of WNV emphasizes the potential role of migratory birds in introducing these in Europe [20].

As winter approaches in northern latitudes, billions of birds travel every year from their European breeding grounds to warmer areas in Africa [21] covering thousands of kilometers along the Palaearctic-African flyway. This migration system connects the two continents and their ecosystems twice every year [22], including origin sites, stopover areas, and destination sites. Given that birds can carry and spread pathogens [23], including viruses that affect humans and livestock [24], there is significant interest in understanding the potential role of migratory birds in WNV outbreaks in Europe and Africa.

In this study, we adopted a One Health approach to identify potential avian reservoirs for the intercontinental dispersal of WNV between the Western Palaearctic and the Afrotropical region. We examined how bird species distribute themselves during breeding and wintering seasons, identifying shared distribution patterns known as chorotypes [25]. By comparing the geographic distribution of bird chorotypes with the distribution of the WNV on both continents, we aimed to prioritize bird species for further investigation regarding their potential role in introducing new WNV strains.

If bird chorotypes are objectively detected, then it is not necessary to invoke a different role in the spreading of the virus for each species. Furthermore, the role of species in the same chorotype is the same irrespective of the habitat they use. In this way, the interpretation of the interconnection patterns between the two biogeographic realms may be more parsimonious and comprehensible.

Given that WNV is a transboundary disease of international concern, we studied this virus using an approach that relates the broad-scale pattern of human cases with the biogeographic re-arrangement of migratory birds in the breeding and wintering seasons, thus relating human health and natural processes as advocated by the One Health approach. By integrating information from various disciplines, such as virology, macroecology, and epidemiology, we can enhance our One Health understanding of WNV transmission dynamics to develop effective prevention and control strategies.

2. Methods

2.1. Database

The study area comprised the Western Palaearctic and Afrotropical realms, which included the Afro-Palaearctic migratory system. From this migratory system, we selected bird species belonging to Orders that are known to be competent reservoirs for WNV, as they develop a high level of viremia [26,27]. Specifically, we focused on five Orders: *Accipitriformes*, *Charadriiformes*, *Falconiformes*, *Passeriformes*, and *Strigiformes* based on previous research [26–31]. Within these Orders, we considered species that migrate between Africa to Europe (**Supplementary Table 1**).

To determine the species distributions, we obtained polygon shapefiles from the International Union for Conservation of Nature's website (IUCN Red List of Threatened Species. http://www.iucnredlist.org). We used the Palaearctic distribution of birds during their breeding season, and their Afrotropical distribution during their wintering period. These distributions were then projected onto a hexagonal grid, with each hexagon representing Operative Geographical Unit (OGU) spanning an area of 7774-km^2.

For the identification of the geographic locations of WNV human cases, we conducted a literature search using databases such as GIDEON [32], Web of Science, Scopus, Google Scholar, and the European Centre for Disease Prevention and Control [33] (ECDC) database. We gathered data from 1937 (when the virus was first described), up until 2020. To facilitate comparison, we projected the WNV cases onto the same hexagonal OGUs used for bird, ensuring consistency in the spatial scale of analysis.

2.2. Chorotypes and data analysis

Chorotypes are particular distribution patterns significantly shared by a group of species, or the distribution pattern of a single species that does not overlap significantly with any other chorotype. These patterns may be influenced by historical, geographical and/or ecological factors, but are not necessarily tied to a specific ecosystem [25,34]. Chorotypes may overlap, but their degree of overlap is no greater than would be expected by chance. In our study, we generated separate chorotypes for the breeding and wintering areas within the Western Palaearctic and Afrotropical realms, respectively.

To identify chorotypes, we built a presence-absence matrix for the bird species identified, which were hierarchically classified using the Baroni-Urbani & Buser similarity index [35] and the Unweighted Pair Group Method with Arithmetic Mean Agglomerative Algorithm (UPGMA [36]). We assessed the resulting classification dendrogram for statistical significance using RMacoqui 1.0 software (http://rmacoqui.r-forge.r-project.org) [25]. Chorotypes were defined as groups of species with either a significant positive internal homogeneity (IH) value either 1 or positive and significant ($P < 0.05$). The resulting chorotypes were mapped, illustrating their species richness in each OGU within the study area.

In the Western Palaearctic, where WNV human cases have been well studied and documented in the scientific literature and ECDC database, we tested the association between the breeding season chorotypes and the distribution of WNV cases. Logistic regression analysis was employed, with the presence/absence of WNV human cases as the dependent variable, and the species richness of chorotypes as the predictor variables. However, for Africa, where the occurrence of WNV cases in humans is likely to be under-recorded [7] due to symptom confusion with other diseases like malaria and typhoid fever [37] we used a risk model for WNV disease. This model determined the environmental favorability for WNV infections in Africa, incorporating anthropic (e.g., infrastructures or agriculture) and non-anthropic variables (e.g., climate, topography and ecosystems). The ecosystem variables comprised wetlands of international importance for birds (Ramsar sites) and land cover (**Supplementary Table 2**). We examined the correlation between this level of environmental favorability and the wintering Afrotropical chorotypes using Spearman correlations. Although our analyses did not explicitly include vectors, due to the study scale, they are implicitly present in our models through the distribution of WNV human cases (which implies the presence of competent mosquitoes in the area). At the intercontinental level there are different likely mosquito species linked to WNV transmission, which have a widespread distribution in the two biogeographic realms (**Supplementary Fig. 2 and Supplementary Table 3**). For this reason, we focused on birds, considering that the presence of mosquitoes is not a limiting factor for the spread of the virus at this geographic scale.

To build the risk model for WNV disease in Africa, we first performed a univariate logistic regression of the recorded distribution of WNV disease in Africa on each explanatory variable separately. We controlled for multicollinearity among environmental variables by calculating Spearman correlation coefficients and choosing the most explanatory variables within highly correlated explanatory factors (correlation >0.8), based on the significance of the Rao's score test of each univariate model [38]. We limited the increase in type I error caused by the number of variables analyzed by addressing a False Discovery Rate control [39]. Using a multivariate stepwise logistic regression, a machine learning algorithm [40], variables were added to an initial null model if their inclusion significantly improved the regression. We assessed multicollinearity effects in the model by calculating the variance inflation factor (VIF) for each variable, with VIF values >5 indicating strong multicollinearity. Careful examination of coefficients was required for variables with high VIF values [41]. Probability values resulting from the logistic regression were transformed into favorability values (ranging from 0 to 1) using the Favorability Function [42], representing deviations from the expected chance of WNV outbreaks and identifying localities with environmental conditions associated with outbreak occurrence. This modelling approach conforms to standard modelling protocols [43].

Finally, we tested whether chorotypes were significantly related to the distribution of cases derived from the two main WNV lineages in the Afro-Palaearctic (lineages 1 and 2), considering the Western Palaearctic and the Afrotropical realms separately. Logistic regressions were employed with the presence of cases for a specific lineage as the dependent variable, and the species richness of a chorotype as the independent variable. The presence of lineages circulating in different countries within the study area, as well as the lineages implicated in the human cases (**Supplementary Fig. 1**) were considered for the dependent variable, and these were referred to at the OGU resolution level.

3. Results

In the Western Palearctic, although human cases of WNV are prevalent throughout the Mediterranean basin, historically the highest incidence of the virus has been observed in Eastern Europe and southern Russia, particularly in the Black Sea and Caspian Sea basins (Fig. 1**.A**). In the Afrotropical realm, the environmental model identified high-risk areas in countries surrounding Lake Victoria, where WNV was first discovered, as well as in eastern South Africa. Additionally, continuous risk areas were identified along the Sahel belt, particularly in West Africa, which serves a wintering grounds for many birds breeding in Western Europe winter (Fig. 1**.B**). Anthropic variables played a crucial role in explaining the model, with variables such as population density (Wald test = 22.029), cropland density (Wald test = 15.191), distance to road (Wald test = 13.609) and forest loss (Wald test = 8.420) significantly associated with the distribution of human WNV infections in Africa. Climatic and ecosystem variables were also significantly related to the occurrence of WNV infections: precipitation (Wald test = 12.503), proximity to Ramsar sites (Wald test = 12.127) and proximity to rivers (Wald test = 6.763) (**Supplementary Table 4**). All variables that were part of included in the model had a VIF lower than 5, indicating a low correlation between the independent variables (**Supplementary Table 4**).

3.1. *Chorotypes*

Birds were classified, during their breeding season in the Western Palaearctic, into nine chorotypes (**Supplementary Fig. 3**). In their wintering areas in Africa, these birds were clustered into 14 chorotypes (**Supplementary Fig. 4**). The change of season represents a biogeographic reorganization of the migrant bird communities, as species composition of the breeding chorotypes differs from that of the wintering chorotypes (Fig. 2).

Out of the nine breeding chorotypes detected, seven of them were significantly associated with WNV infections, suggesting that the species within these chorotypes could contribute to the dispersal and amplification of the virus in Europe. Similarly, among the wintering chorotypes in Africa, nine were significantly correlated with the risk of WNV disease, indicating their potential as WNV amplifiers in Africa. Among the initial 123 migrant species, 61 species were found in chorotypes related to WNV infections in both continents (**Supplementary Table 5**), belonging to the orders *Accipitriformes* (12 species), *Falconiformes* (4 species), *Strigiformes* (1 species), *Charadriiformes* (14 species), and *Passeriformes* (30 species).

In the Western Palaearctic, all chorotypes associated with WNV infections were linked to both 1 and 2 lineage strains, except for the Mediterranean chorotype (*b_C6*), which was exclusively associated to lineage 1. In the Afrotropical realm, with the exception of a coastal chorotype composed of littoral birds (*w_C6*), all other chorotypes were associated to at least one lineage. The chorotype around Lake Victoria (*w_C11*) was exclusively associated with lineage 2. Two chorotypes (*w_C3* and *w_C7*), primarily located above the Equator, were associated solely to lineage 1. The chorotypes associated with lineages 1 and 2 in the Western Palaearctic consisted of species that were also linked to both lineages in the Afrotropical region. In addition, the species within the breeding chorotype *b_C6* were exclusively associated with lineage 1 during the wintering season, specifically to the Afrotropical chorotypes *w_C3* and *w_C7* (Fig. 2).

4. Discussion

Previous studies have suggested that migratory birds play a role in the spread of WNV in America [11] and in Europe [44]. Researchers have classified birds based on their migration routes [11] and movement types (short or long-distance migrants) [45] to predict the spread of the virus. Birds that winter in sub-Saharan Africa and breed in Europe have been identified as potential candidates for introducing the virus to Europe [46,47]. In this study, we go a step further by using species chorotypes to analyze the distribution patterns of migratory birds in Africa and Europe and over the annual cycle. Our analysis identified 61 migratory species as potential vectors of WNV across both continents, with evidence that 44 species being infected by the virus: 35 of them detected in Europe, 3 in Africa, and 6 in both Africa and Europe (**Supplementary Table 5**). Infection has been detected in all species of *Falconiformes* and *Strigiformes* on our list, as well as in three-quarters of the *Accipitriformes* and *Passeriformes*, validating our results. In the case of the *Charadriiformes*, 50% of the species in our list have been reported to have been in contact with the virus and, thus, are potentially involved in the virus's intercontinental spread, although bias in the testing and reporting, both in species and numbers, may underestimate the role of this group.

Passeriformes and *Charadriiformes* are the groups most likely involved in the amplification and spread of the WNV in wetlands (Ramsar sites in our case). These wetlands are important sites for the breeding, wintering and migration of waterbirds, and also suitable areas for mosquitoes [48]. However, in forested areas, *Accipitriformes*, *Falconiformes*, *Strigiformes* and *Passeriformes* are the main species groups associated with the WNV cycle. Deforestation, an important variable explaining WNV cases, can impact bird communities and increase contact between vectors and avian reservoirs [49]. Forest loss may facilitate exchanges between human and zoonotic cycles as open areas are favored for human settlements [50]. The relationship between deforestation and the occurrence of zoonotic outbreaks has already been suggested [51], and should be further investigated for WNV.

There is a clear north-south gradient in the incidence of WNV in Europe. Northern Europe is not heavily affected by WNV infections yet though this may change since the virus is already endemic in southern regions of Europe. Whereas countries in the south have suffered recent WNV outbreaks, the dispersal of WNV to northern European countries may become more frequent. Antibodies to WNV have been detected among different migrating birds in different countries well in advance of

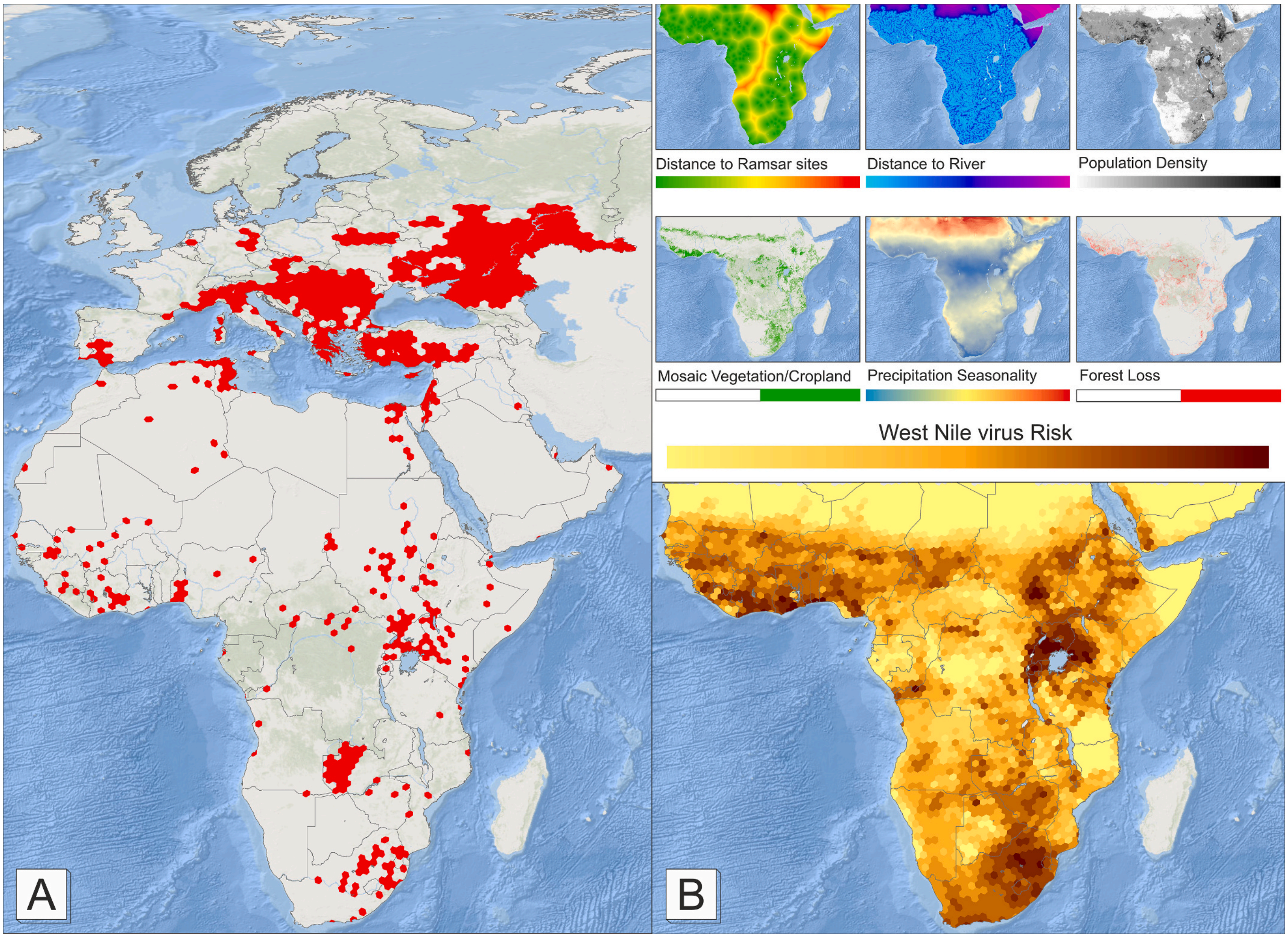

Fig. 1. West Nile virus in the Afro-Palaearctic. (A) Afro-Palaearctic distribution of WNV human cases. (B) WNV risk model in the Afrotropical realm and the cartographical representation of some explicative variables related to WNV cases.

any cases in horses or humans, e.g. in Sweden in 2005–2006 [52], and Germany in 2005–2007 and 2009–2011 [53,54]. From 2014 to 2016, neutralizing antibodies were found in migratory and resident birds in Germany, but predominantly in migratory species [55]. A similar pattern was found in countries with recurrent WNV outbreaks, and where long-distance migratory birds have a higher prevalence of WNV neutralizing antibodies than resident or short-distance migratory birds (France [56] e.g., Italy [57] and Spain [47]). Although presence of antibodies indicate previous exposure to WNV and no current virus infection and no dispersal of the virus is expected, these results highlight that the main exposure to WNV occurs during their stay in sub-Saharan Africa [47,56].

Long-distance migratory birds may play a crucial role in the initial spread of WNV spread from Africa to Europe, while resident or short-distance migratory birds, may amplify and disperse the virus locally. This role has been proposed for the Eurasian Blackbird (*Turdus merula*), since it is a preferred host for *Culex pipiens*, an important vector of WNV, and can exhibit high antibody titers [58,59]. Western Europe receives many avian species that follow the western migratory route [60] and spend the boreal winter in West Africa (*w*_C3 and *w*_*C7*), a high risk region highlighted in our model. Moreover, in Africa, chorotypes *w*_*C3* and *w*_*C7* were exclusively associated with lineage 1. Species included in these chorotypes formed the chorotype *b*_*C6* in Europe, which was also exclusively associated to lineage 1. On the other hand, Eastern European countries are greatly affected by the virus, where WNV lineage 2 was first discovered [14]. This region receives migratory species from the Eastern and southernmost parts of Africa, two hot spots areas of WNV risk, and covered by *w*_*C1* and *w*_*C4* chorotypes. Moreover, the predominant lineage in these African areas is lineage 2. This supports the existence of a bird-mediated relationship between WNV infection risk areas in Africa and Europe.

The role of migratory birds in long-distance pathogen dispersal may also be facilitated by a reduced immune response to pathogens. To begin with, Palearctic breeders who colonized from Africa, including migrants, have reduced diversity in some immune genes associated with pathogen recognition compared to African residents [61]. Additionally, during migration, immune function can be compromised due to physiological stress or energetic trade-offs [62], potentially leading to higher virus titers and recrudescence of latent infections in migratory birds [63]. The arrival of many birds with a suppressed immune system in Europe may contribute to greater amplification of the virus compared to resident birds. This effect is difficult to separate from those due to the increase in mosquito populations in spring, when long-distance migratory birds arrive at their breeding grounds.

Mosquitoes, particularly those of the genus *Culex,* are the main vectors of WNV, but other mosquito genera known to have transmission capacity found throughout the study area, such as *Ochlerotatus*, *Mansonia* and *Aedes,* although the ecological role of *Aedes* in the transmission is still unknown (**Supplementary Fig. 2 and Supplementary Table 3**). Our large-scale analyses may be affected by small-scale processes, such as the diversity and abundance of WNV transmission-competent mosquitoes. However, the lack of fine-scale distribution and

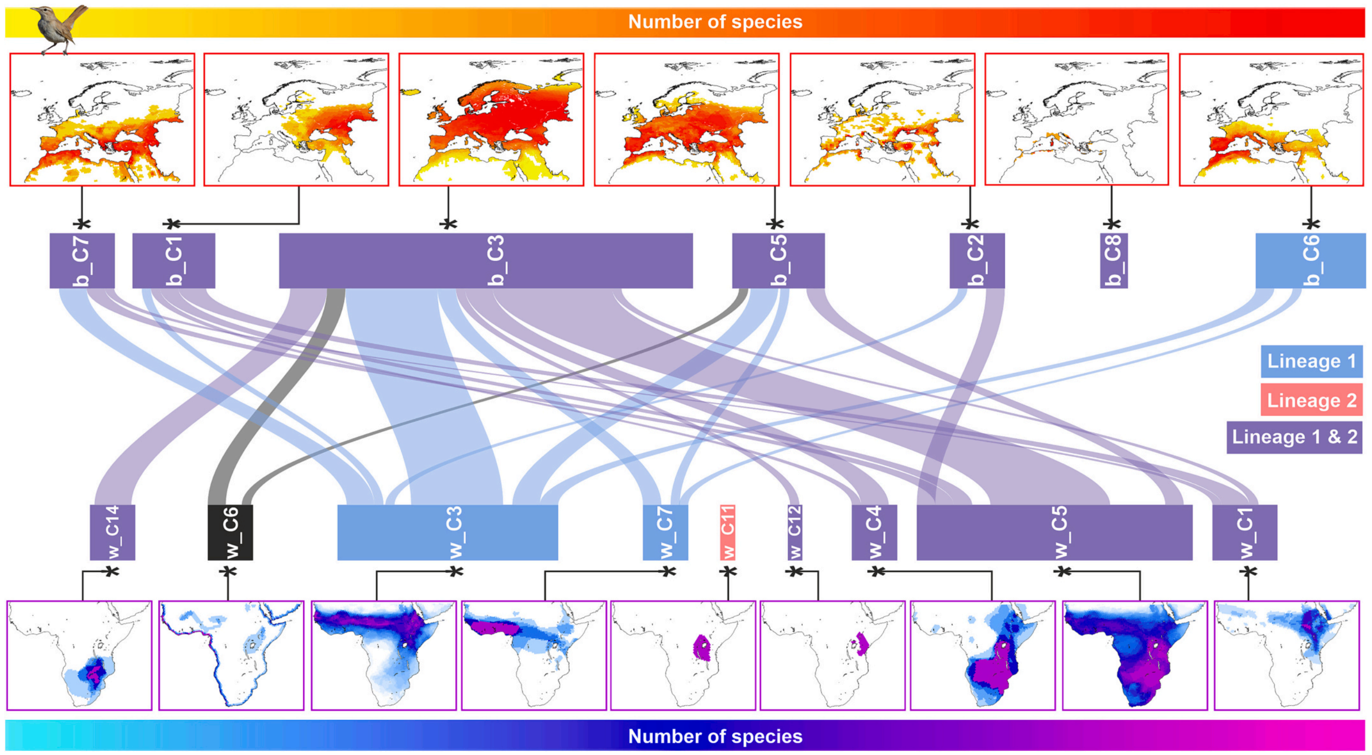

Fig. 2. Connection of West Nile virus cases and their lineages between Africa and Europe via migratory birds. Breeding (b_Cx) and wintering bird chorotypes (w_Cx) significantly associated with human cases of WNV disease, both in the Western Palaearctic (top maps) and in the Afrotropical realms (bottom maps). Chorotypes are groups of species with statistically similar distribution patterns, irrespective of the habitat they use. Chorotypes may overlap, but their degree of overlap is no greater than randomly expected. The size of the rectangular boxes indicates the number of species forming part of the chorotype. The width of bands connecting Western Palaearctic and Afrotropical chorotypes represents the number of species that are shared by them. Boxes, and bands, are colored according to the WNV lineage associated to the chorotype: blue for lineage 1, red for lineage 2, and violet for lineages 1 and 2. The black box, and its black bands, is the chorotype and its species significantly associated to human cases of WNV disease, but not associated to any lineage. The bird species that belong to each chorotype can be seen in **Supplementary Table 5**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

abundance data at our study scale precludes their inclusion in the analyses. However, the role of birds as key components in the intercontinental translocation of the virus is not affected by this limitation, as mosquitoes are not a limiting factor at the geographic scale of the study. Our study would also benefit by the inclusion of relative abundance of migratory birds during migration throughout the study area, mainly in wetlands, but this information is also lacking for most of the OGUs.

Climate change can alter the transmission dynamics of diseases by affecting the virus, vectors and reservoirs. Rising temperatures can increase virus replication rates and expand the range of vectors to higher altitudes and latitudes [64]. Migratory species respond to climate change by shifting migratory routes and phenology in response to temperature and the resource availability [65]. This can lead to higher exposure to the virus in previously unaffected areas, as infected birds change their distribution. Identifying the species in virus translocation can help anticipate changes in their distributions and, therefore changes in disease distribution. It can also aid in identifying the introduction of new WNV and other flavivirus strains.

5. Conclusions

We have applied a One Health approach, which considers the interconnections of animals, ecosystems and human health. By combining the expertise of ecologists/biologists, virologists and biogeographers, we aim to tackle the challenges posed by emerging diseases. This research represents the first attempt to establish a connection between WNV cases on different continents through bird migration, considering the spatial distributions of migratory species during their breeding and wintering seasons. By identifying potential reservoir species that share distributions throughout the period when WNV outbreaks occur, as well as understanding how bird communities reorganize from season to season, we can adopt an intercontinental approach to disease management. To effectively address WNV outbreaks in Europe and Africa, it is crucial to implement a coordinated One Health surveillance approach that employs cross-sectoral and cross-disciplinary strategies. Recognizing that billions of birds connect these geographically distant regions twice a year, such approaches will greatly enhance preparedness efforts.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2023.100585.

Author contributions

Conceptualization: J.-M.G.-C., RR and A.-R.M. Methodology: J.-M. G.-C., R.R. and. J.O. Data analysis: J.-M.G.-C. Supervision of analysis: J.O., R.R. and A.-R.M. Writing-original draft: J.-M.G.-C. Final review & editing: J.-M.G.-C., A.-R.M., J.O., J.F., J.E.F. and R.R.

Declaration of Competing Interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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