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1 **Spatio-temporal Genotype Replacement of H5N8 Avian Influenza Viruses Contributed to**  
2 **H5N1 Emergence in 2021/2022 Panzootic**

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33 Running Head: Spatio-temporal spread of H5Ny in 20-22 pandemic

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38 Abstract: 156 words. Main text: 6787 words.

39 **ABSTRACT**

40 Since 2020, clade 2.3.4.4b highly pathogenic avian influenza (HPAI) H5N8 and H5N1 viruses  
41 have swept through continents, posing serious threats to the world. Through comprehensive  
42 analyses of epidemiological, genetic, and bird migration data, we found that the dominant  
43 genotype replacement of the H5N8 viruses in 2020 contributed to the H5N1 outbreak in the  
44 2021/2022 wave. The 2020 outbreak of H5N8 G1 genotype instead of G0 genotype produced  
45 reassortment opportunities and led to the emergence of a new H5N1 virus with G1's HA and MP  
46 genes. Despite extensive reassortments in the 2021/2022 wave, the H5N1 virus retained the HA  
47 and MP genes, causing a significant outbreak in Europe and North America. **Furtherly, through**  
48 **the wild bird migration flyways investigation, we found that the temporal-spatial coincidence**  
49 **between the outbreak of H5N8 G1 virus and the bird autumn migration may have expanded H5**  
50 **viral spread, which maybe one of the main drivers of the emergence of 2020-2022 H5 panzootic.**  
51 ~~We further found that viral evolution in poultry of Egypt and surrounding area and bird migration~~  
52 ~~from Russia-Kazakhstan region to Europe are important drivers in the emergence of the 2020-~~  
53 ~~2022 H5 panzootic.~~

54 **IMPORTANCE**

55 **Since 2020, highly pathogenic avian influenza (HPAI) H5 subtype variants of clade 2.3.4.4b have**  
56 **spread across continents, posing unprecedented threats globally. However, the factors promoting**  
57 **the genesis and spread of H5 HPAI viruses remain unclear. Here we found that the spatio-temporal**  
58 **genotype replacement of H5N8 HPAI viruses contributed to the emergence of H5N1 variant that**  
59 **caused the 2021/2022 panzootic, and viral evolution in poultry of Egypt and surrounding area and**  
60 **autumn bird migration from Russia-Kazakhstan region to Europe are important drivers of the**

61 emergence of the 2020-2022 H5 panzootic. These findings provide important targets for early  
62 warning and could help control the current and future HPAI epidemics.

63 **KEYWORDS** Avian influenza virus (AIV), H5N1, H5N8, genesis, spread, bird migration

## 64 MAIN TEXT

65 The ongoing avian influenza virus (AIV) H5N1 outbreak is the largest ever recorded and is  
66 affecting wild birds, poultry, mammals, and humans across multiple continents. In Europe, the  
67 2021-2022 epidemic has resulted in a total of 2,520 HPAI outbreaks in poultry, with 50 million  
68 birds dead or culled, 227 findings in captive birds, 3,867 HPAI virus detections in wild birds, and  
69 1 human case(1). In the United States, between January 2022 and 15 March 2023, more than 58.6  
70 million domestic birds were affected, along with 6,444 wild bird detections and 1 human case(2,  
71 3). The origin of the virus and how to control its spread are urgent concerns in light of this  
72 unprecedented outbreak.

73 Since the first isolation of the HPAI virus [A/Goose/Guangdong/1/96 (H5N1)] belonging to  
74 GS/GD lineage was detected in China in 1996, H5 HPAI has undergone rapid genetic divergence  
75 and genetic reassortment, resulting in the emergence of numerous clades and subclades(4–6). Over  
76 the years, there have been six waves of intercontinental transmissions caused by the GS/GD  
77 lineage viruses through migratory birds(7–10). In 2005-2006, clade 2.2 H5N1 virus spread out  
78 from Qinghai Lake of China to other countries in Europe and Africa(11, 12). In 2009-2010, clade  
79 2.3.2.1 H5N1 virus affected Asia and Europe(13). Subsequently, clade 2.3.4.4a and 2.3.4.4b of  
80 H5N8 viruses caused multiple waves of worldwide epidemics in 2014-2015 and 2016-2021,  
81 respectively(10, 14, 15). Following that, a novel H5N1 virus of clade 2.3.4.4b has emerged,  
82 circulating in Europe, North America, South America, Africa, and Asia(16, 17), causing the most  
83 lasting and devastating threats to the world.

84 The clade 2.3.4.4b H5N8 outbreaks were first identified in Qinghai Lake of China during the  
85 epidemic season of 2016/2017, and rapidly spread to almost all of Eurasia(15). However, in 2020-  
86 2021, the clade 2.3.4.4b H5N8 caused an unexpected epidemic peak(18, 19). In Europe alone, the

87 virus caused 1,298 outbreaks in poultry, affecting 229 million domestic birds, 85 detections in  
88 captive birds, and 2,294 HPAI events in wild birds(20). Moreover, during this wave, the World  
89 Health Organization (WHO) reported the first human infections with HPAI H5N8 viruses(21). In  
90 the following epidemic season of 2021/2022, the H5N1 subtype replaced the H5N8 and triggered  
91 a larger panzootic(16, 22), affecting more species. However, there are still some key questions  
92 related to these outbreaks that remain unclear. Specifically, it is unclear how the successive  
93 outbreaks of H5N8 and H5N1 are related, as well as what factors contribute to the two outbreaks.  
94 Addressing these questions is significant for the control and prevention of the potential pandemic.  
95 In this study, we collected all available viral genome sequences of H5N8 and H5N1 that were  
96 sampled between October 2016 and September 2022, along with other related sequences before  
97 this time period. We then used Bayesian phylodynamic modeling to investigate the genetic  
98 evolution and spread of HPAI H5N8 and H5N1 viruses during the epidemic seasons of 2020/2021  
99 and 2021/2022, and incorporated epidemiological data and bird migratory data to independently  
100 assess the consistency.

## 101 **RESULTS**

### 102 **H5 HPAI viruses initiated the biggest outbreak in 2020-2022 after a quiet period**

103 Our analysis of the epidemiological data from FAO EMPRES-i revealed four primary subtypes of  
104 H5N1, H5N2, H5N6, and H5N8 viruses prevalent in Europe, America, Asia, and Africa. H5N1  
105 and H5N8 caused three epidemics since 2016 (Fig. 1A and F), with H5N8 causing the first wave  
106 in Europe, Africa, and Asia from October 2016 to September 2017 (2016/2017 Wave). In  
107 2017/2018, 2018/2019 and 2019/2020, the period looks like a quiet stage as there weren't massive  
108 reported outbreaks. After that, the H5N8 initiated the second wave in same continents in

109 2020/2021. However, another subtype of H5N1 replaced the H5N8 virus in the following  
110 2021/2022 wave, causing a wider outbreak across Eurasia, Africa, and North America (Fig. 1B-  
111 E). Prior to the 2020/2021 wave, there was a low-level prevalence caused by H5N8 virus in  
112 2019/2020 wave (Fig. 1A and C). Among the three major epidemics of 2016/2017, 2020/2021 and  
113 2021/2022 waves, there were outbreaks occurred in domestic poultry and wild birds, with wild  
114 birds contributing to a greater percentage of the outbreaks in the later waves (Fig. 1F, Table S1).  
115 A more detailed case and death data were further summarized by waves, showing a growth trend  
116 across waves in wild birds (Fig. 1G). Human cases infected with HPAI H5Ny also peaked in 2020-  
117 2022 (Fig. 1H). The results indicated that the 2021/2022 wave of H5N1 was the largest and most  
118 devastating HPAI epidemic. Our analysis raises questions about why the H5N8 virus was able to  
119 re-initiate a big outbreak in 2020/2021 and whether this outbreak contributed to the subsequent  
120 H5N1 panzootic.

121 **The genomes of H5N8 and H5N1 viruses in 2020-2022 are divergent from other clade 2.3.4.4b**  
122 **viruses**

123 To explore the factors inducing the 2020/2021 H5N8 and 2021/2022 H5N1 waves, a phylogenetic  
124 analysis of HA gene in clade 2.3.4.4b H5Ny viruses was performed using a full genome dataset  
125 composed of 3781 HPAI H5Ny viruses collected around the world between 2016 and 2022 (Fig.  
126 2A). We further calculated the pairwise identity of eight gene segments of all sequences and  
127 visualized the results using heatmap (Fig. 2B-I). Our analysis of the reconstructed phylogenetic  
128 tree of the HA gene revealed that the 2016/2017 wave of HPAI H5N8 virus had high gene  
129 homology (98.75%, Table S2) but different geographical distributions. Some were mainly found  
130 in Asia and Africa, while others mainly in Europe and Africa (Fig. 2A). The HA gene of 2019/2020  
131 and 2020/2021 waves of H5N8 viruses formed from two distinct groups (Fig. 2A). The 2019/2020



132 viruses diverged from those isolated in Asia and Africa during 2016/2017 wave (with 97.2%  
133 identity, Table S2), and 2020/2021 viruses mainly diverged from those isolated in Europe and  
134 Africa in 2016/2017 wave (with 97.38% identity, Table S2). However, both two groups have a  
135 relatively long leading branch connecting the viruses of 2016/2017 wave. Apart from HA, the two  
136 groups of viruses also have different sequences from those of 2016/2017 wave in other seven  
137 segments (76.79% to 97.83% identity, Table S2, Fig. 2B-I). Therefore, a potential hypothesis is  
138 that the 2019/2020 and 2020/2021 waves were caused by two new H5N8 variants differently from  
139 the initial clade 2.3.4.4b viruses. However, the H5N1 virus of the 2021/2022 wave had high  
140 identities with those of H5N8 2020/2021 wave, in HA (98.29%, Table S2, Fig. 2E) and MP  
141 (98.77%, Table S2, Fig. 2H) genes, but low identities (68.85%-95.14%, Table S2) with those  
142 viruses in other genes (Fig. 2B-I). So, the consecutive emergent 2020/2021 H5N8 and 2021/2022  
143 H5N1 viruses had divergent genomes from those of the previous waves, but they shared similar  
144 HA and MP genes with each other.

145 **H5N1 virus in panzootic acquired the HA and MP genes from H5N8 HPAI viruses and other**  
146 **six genes of diverse LPAI viruses**

147 To investigate the genetic origins of H5N1 virus in the 2021/2022 wave, we constructed  
148 phylogenetic trees using data from eight segments (Fig. S1-S9). The results showed the earliest  
149 H5N1 virus strain isolated in Europe and in North America (A/Eurasian  
150 wigeon/Netherlands/1/2020 and A/Fancy chicken/NL/FAV-0035/2021, respectively) were  
151 grouped together with the H5N8 virus of the 2020/2021 wave in Europe in the trees of HA and  
152 MP genes (Fig. S1, S5 and S8), but were separated from the H5N8 virus of the 2019/2020 wave  
153 and earlier ones (Data S1 and Fig. 2). In the trees of the other six genes, the two viruses exhibited  
154 the closest genetic relationship with diverse LPAI wild bird strains from Eurasia. Subsequently,

155 some H5N1 viruses isolated in the United States underwent further reassortment with the local  
156 LPAI strains. Specifically, all eight segments of the early H5N1 strains of North America clustered  
157 with European H5N1, while late North American H5N1 strains were rooted with North American  
158 LPAIVs, especially in PB2 and NP segments (Fig. S2-S9). North American-like PB2 and NP  
159 strains were predominantly found in the north-central United States, such as Minnesota, South  
160 Dakota, North Dakota, and Iowa in March and April of 2022, whereas other strains with European-  
161 like genes were distributed mostly in March of the eastern seaboard of the States, such as Maine  
162 and North Carolina (Fig. S2 and S6). Overall, the results suggested that European H5N1 strains  
163 first arrived on the east coast of the North American continent and then gradually spread  
164 westwards, acquiring local LPAIV genes to form more reassortants. Notably, regardless of whether  
165 they were European H5N1 or later North American H5N1 strains, they consistently retained the  
166 HA and MP genes originating from the 2020/2021 H5N8 virus, suggesting these two genes might  
167 have contributed to the emergence and further transmission of the H5N1 virus.

#### 168 **Dominant genotype change in H5N8 virus drives the 2020/2021 wave**

169 To identify the specific H5N8 virus that contributed the HA and MP genes to the H5N1 virus, we  
170 analyzed the genotypes of the earlier H5N8 virus isolated during the 2019/2020 and 2020/2021  
171 waves (Fig. 3A). The sequences were divided into 3 to 12 distinct phylogenetic monophyletic  
172 groups for each gene segment (Fig. S10 and S11) and 22 genotypes were identified for all HPAI  
173 H5N8 viruses, including two major genotypes (G0, G1, dominant circulate genotypes), nine minor  
174 genotypes (reassortant genotypes of major), and eleven other transient genotypes (with one  
175 sampled isolate, Fig. 3A, Table S3). Notably, we found that the strains providing the HA and MP  
176 genes for the H5N1 virus belonged to the H5N8 G1 genotype (Fig. S5 and S8, Data S1).

177 Temporal dynamic analysis revealed that G0 and G1 genotypes have successively dominated the  
178 HPAI H5N8 epidemic in the Eurasian continent since 2020 (Fig. 3B and C, Fig. S12). G0  
179 dominated in the first phase, from January 2020 to June 2020, primarily affecting domestic poultry  
180 in the middle and eastern Europe (Fig. 3B and D, Fig. S12). However, during the low epidemic  
181 period from June to August 2020, the distinct genome set of G1 replaced G0 and dominated the  
182 following 2020/2021 wave (Fig. 3B and C). G1 affected both domestic poultry and wild birds for  
183 a longer time, from August 2020 to December 2021, and showed a higher epidemic scale in terms  
184 of increased number of outbreaks, species of wild birds and geographic distribution (Fig. 3B and  
185 E, Fig. S12). Furthermore, reassortment during this epidemic generated the diversified G1-like  
186 genotypes (Fig. 3B and C, Table S3), increasing the likelihood of G1 providing genes to the novel  
187 H5N1 virus. The dominant replacement of G0 by G1 was a prerequisite for the 2021/2022 H5N1  
188 wave.

189 **H5N8 virus circulating in Egypt and surrounding area since 2018 identified as the ancestor**  
190 **of the dominant G1 in 2020/2021 wave**

191 To investigate how the H5N8 re-emerged and became dominant in the Eurasian continent after a  
192 long quiet period, we compared the evolution history of the two distinct genotypes of G0 and G1  
193 using phylogeographic continuous diffusion model. Our findings for G0 revealed the most recent  
194 common ancestor (MRCA) of all gene segments emerged in Central Europe (Fig. S13A-H).  
195 However, the most recent common grand ancestor (MRCGA, first recent ancestor node of MRCA)  
196 had six segments originating from South Africa or Western Africa (PB2, PA, HA, NA, MP, NS,  
197 Fig. S13 A, C-D, F-H), and two from Eurasia (PB1, NP, Fig. S13B and E).

198 The inferred time of MRCA (TMRCA) for the genotype G0 was October 2019 [95% HPD: August  
199 2019 to November 2019], while the inferred time of MRCGA (TMRCGA) was February 2019

200 [November 2018 to May 2019] (Fig. S13I). The likely hosts of MRCA and MRCGA for almost  
201 all gene segments were domestic *Anseriformes* or domestic *Galliformes*, except for PB1 and NP  
202 genes, which the MRCGA were inferred to have waterfowls as hosts (Fig. S13J). Therefore, it is  
203 possible that the HPAI H5N8 viruses circulating in Western African and South African poultry  
204 provided the backbone genes (6 of 8 gene segments) for the genotype G0 at the beginning of 2019.  
205 The ancestor of genotype G0 emerged by reassorting the PB1 and NP gene segments of LPAI  
206 viruses from Eurasian waterfowls at the wintering sites of middle Europe around October 2019  
207 (Fig. S13K). Following that, the G0 strain A/turkey/Poland/23/2019 was firstly isolated in Poland  
208 and caused a peak infection from January to April in 2020.

209 The location of MRCA for all segments of G1 was traced to a larger area around the border of  
210 Russia-Kazakhstan, which acted as a crossroads between Europe and Asia (Fig. S14A-H).  
211 However, nearly all segments of MRCGA for G1 were estimated from Egypt and surrounding area,  
212 especially the PB1, PA, HA and NP segments are mainly located in Egypt (Fig. S14A-F, S14H).  
213 The only exception is MP segment, which was most likely located in northwest China (Fig. S14G).  
214 The TMRCA for G1 was estimated to be March 2020 [December 2019 to May 2020], similar to  
215 G0. But the TMRCGA for G1 was estimated to be much earlier, in May 2018 [May 2017 to  
216 November 2018] (Fig. S14I). Except for the MP gene, whose host is unclear (domestic  
217 *Anseriformes* or waterfowl), the other segments of MRCA and MRCGA are all presumed to be  
218 domestic *Anseriformes* or domestic *Galliformes* (Fig. S14J). Similarly, it is possible that the HPAI  
219 H5N8 virus endemic in poultry of Egypt and surrounding area provided the backbone genes (7 of  
220 8 gene segments) for the genotype G1. The ancestor of genotype G1 emerged at the wintering sites  
221 along the Asia-Europe border around December 2019, by reassorting MP gene segment of HPAI

222 H5N8 viruses from Xinjiang, China (Fig. S14K). Then, the first G1 strain A/chicken/Iraq/1/2020  
223 was isolated in Iraq in May 2020 and caused an outbreak peak from December 2020 to April 2021.  
224 Thus, in the “quiet” period, H5N8 viruses kept evolving and spreading. Both G0 and G1 were  
225 probably generated by reassortment with genes from poultry and wild birds in the autumn of 2019.  
226 Their poultry-origin genes are both from Africa, but those of G0 are from West and South Africa,  
227 while those of G1 are from Egypt in North Africa and surrounding area. G0 emerged in middle  
228 Europe, while G1 emerged in the Asia-Europe border. These results suggest that Egypt and  
229 surrounding area is an important source for genetic diversification of avian influenza viruses, from  
230 where the ancestor G1 virus was generated as early as 2018, which then spread to neighboring  
231 continents of Asia and Europe through migratory birds.

### 232 **Temporal-spatial coincidence between outbreaks and bird autumn migration expanded H5** 233 **HPAI viral spread**

234 To investigate the factors influencing the spread of H5 HPAI virus, we examined the impact of  
235 migratory birds, given their geographical relationship to the viral gene source and epidemic scale.  
236 Using continuous phylogeographic analysis, we reconstructed the spatial-temporal spread routes  
237 of G0 and G1 H5N8 genotypes. We then collected 108 species of migratory birds which were  
238 reported to have been infected by HPAI H5N8 viruses, including 29 species with detailed  
239 migration data (Table S4). The migratory flyways, breeding sites, and wintering sites of these birds  
240 were collected and summarized in Fig. S15. By analyzing the collected spread routes, migration  
241 patterns, as well as sampling locations of viruses, we gained a unique perspective on the role of  
242 migratory birds in the spread dynamic of HPAI H5N8 viruses.

243 As shown in Fig. S15B, the breeding sites of these migratory birds cover most areas of Eurasian  
244 continent, while their wintering sites cover nearly all of Africa continent and southern part of the

245 Eurasian continent. Some areas in Europe, Middle East, and eastern Asia have both breeding and  
246 wintering sites (Fig. S15B). The birds migrate along eight major flyways that connect Europe,  
247 Asia, and Africa, including four flyways from southern to northern Asia, two from Africa to  
248 Europe, one from Africa to Asia, and one from Europe to Asia (Fig. S15B).

249 As shown in Fig. 4A and C, the spatially explicit phylogeographic analysis revealed the inferred  
250 transmission dynamic of genotype G0 and G1 since their MRCA phase. By mapping the wild  
251 bird migration pattern onto the sampling locations of viruses (Fig. 4B and D), we found that the  
252 viral transmission matched well with the flyways of wild birds (Fig. 4). Specifically, for G0 viruses,  
253 ancestral viruses were transmitted from the wintering sites in South Africa and western Africa to  
254 the breeding sites in Europe through northward migration between March and June 2019 (Fig. 4A  
255 and 4B, the two green arrows below). Genotype G0 was produced in Europe in the winter of 2019  
256 (Fig. 4A) and spread to northern breeding areas (Fig. 4B, the green arrows up). G0 viruses initiated  
257 local outbreaks in Europe during the spring of 2020 (Fig. 4A) and were soon spread to the breeding  
258 sites in the Arctic through bird autumn migration (Fig. 4B, the purple arrows). Some of the G0  
259 viruses were transmitted to East Asia (Fig. 4B, the dark red dots) during the autumn migration in  
260 2020 but were not isolated in Europe again (Fig. 4B, no dark red dots there).

261 For G1 viruses, the ancestors of genotype G1 viruses were spread via the routes of Egypt, Middle  
262 East, Kazakhstan, and Russia during the northward migration (Fig. 4C). Some G1 viruses  
263 circulated at the breeding sites near the border of Russia-Kazakhstan, where they spilled over to  
264 nearby poultry and amplified (Fig. 4D, the green arrows and the light blue dots). Other viruses  
265 might have continued north to the Arctic. The time and place of the outbreaks in Russia-  
266 Kazakhstan coincided with the ongoing autumn migration of 2020, which made G1 viruses easily  
267 diffuse into different directions across the Eurasian continent, including westward to Europe and

268 southward to eastern Asia, and caused expanding transmission (Fig. 4D, the purple arrows and the  
269 dark blue dots). From another perspective, discrete phylogeographic analysis of host traits further  
270 confirmed these findings. The first wave of HPAI H5N8 epidemic caused by G0 viruses in the  
271 spring of 2020 in Europe, was mainly restricted in domestic poultry. However, a shift of trunk  
272 probability from domestic poultry to wild birds was observed in the second wave (including G0  
273 and G1 viruses), which was in coincidence with the widely spread of 2020-2021 H5N8 epidemic  
274 (Fig. S16).

275 Therefore, the spring migration of wild birds in 2020 may initiate the spread of G0 from Europe  
276 to the Arctic, while the autumn migration of 2020 may facilitate the spread of G1 from Russia-  
277 Kazakhstan region to Eurasia, resulting in contrasting transmission effects, with G0 contracting  
278 and G1 dispersing. Collectively, the spatial and temporal coincidence between autumn migration  
279 of wild birds and the G1 outbreak greatly promoted wide wider spread, particularly in Europe  
280 during the 2020/2021 wave, as observed in Fig. 5A and B. The extensive outbreak in Europe  
281 further created numerous opportunities for reassortment, leading to the emergence of the H5N1  
282 virus (Fig. 5A and B). In October 2020, the first H5N1 reassortant carrying H5N8 original genes  
283 was detected in wild birds in the Netherlands (Fig. S1-S9), and subsequently, more viruses were  
284 isolated in other European countries, resulting in a larger outbreak (Fig. 5B). By late 2021, the  
285 H5N1 virus had spread further via wild birds from Northwestern Europe to the East coast of North  
286 America (Fig. 5B). Thus, the temporal-spatial coincidence between the outbreak and the bird  
287 autumn migration expanded H5N8 viral spread from Russia-Kazakhstan region to Eurasia and  
288 contributed to the emergence of the H5N1 virus in Europe, which was later introduced to North  
289 America.

## 290 **DISCUSSION**

291 Among multiple clades of H5Ny viruses causing intercontinental spread, the clade 2.3.4.4b H5  
292 subtype HPAI virus caused the longest and most serious epidemic in wild birds and poultry. Here,  
293 we explained the genesis and spread of 2020-2022 H5 HPAI viruses, and revealed the important  
294 factors that contributed to their emergence.

295 During the “quiet” period of 2017/2018, 2018/2019, and 2019/2020, the H5N8 virus had ample  
296 time to evolve into a new variant of G1 with divergent HA and other segments, through interactions  
297 between poultry and wild birds in Egypt and surrounding area as well as Asia-Europe border. This  
298 new variant then spread widely during bird autumn migration. The widespread occurrence of  
299 H5N8 G1 virus, particularly in Europe, facilitated the reassortment of the H5N8 virus with other  
300 subtype viruses from wild birds, resulting in the emergence of the novel H5N1 virus that caused  
301 the 2021/2022 panzootic in Europe and North America. Therefore, the emergence of two new  
302 viruses, the H5N8 G1 virus and the H5N1 virus, led to widespread outbreaks in the two waves of  
303 2020/2021 and 2021/2022. Viral evolution in north-east Africa and middle-east Asia, especially  
304 the poultry of Egypt and surrounding area, as well as bird autumn migration from Russia-  
305 Kazakhstan region to Europe were critical factors initiating the 2020-2022 H5 outbreak.

306 Endemic of AIVs in poultry populations provides a breeding ground for their evolution through  
307 reassortment and mutation. For instance, the prevalence of H9N2 AIVs in chickens in China  
308 enabled the virus to participate in reassortment events, leading to the emergence of the novel H7N9  
309 virus in 2013(23). In this study, we revealed similar consequences resulting from the endemic  
310 epidemic of H5 subtype AIVs in north-east Africa and middle-east Asia, especially the poultry of  
311 Egypt and surrounding area, which served as a gene source for the H5N8 (7 of 8 segments) and  
312 H5N1 (mutated HA segment) viruses during the 2020-2022 outbreak. **Similar findings about the**



313 genetic origins of recently resurgent HPAI H5 epidemics were also reported by Xie et al.(24).  
314 Differently, we here found two genotypes of HPAI H5N8 (G0 and G1 viruses) before the  
315 emergence of H5N1, and the replacement from G0 to G1 is a critical event contributing to the later  
316 emergence of HPAI H5N1, and the spatial-temporal coincidence between the G1 outbreak and the  
317 bird autumn migration may have expanded H5 viral spread.

318 Egypt, being located at the intersection of the Black Sea-Mediterranean and East African-West  
319 Asian Flyways, is a key habitat for a wide variety of waterfowl that connect Africa and Eurasia(25,  
320 26). The country also experiences an annual increase in poultry production, which, coupled with a  
321 relatively rough farming approach, creates fertile ground for the endemicity of highly pathogenic  
322 avian influenza, enabling the disease to persist in the area. The selection pressure brought by  
323 poultry vaccination further complicated the evolution of HPAI virus(27, 28). Phylogeographic  
324 research has revealed that during the 2016-2017 epidemic season, there were at least six  
325 independent introductions of H5N8 virus from the Eurasian continent to Africa, including three  
326 introductions to Egyptian poultry, two introductions to western African poultry, and one  
327 introduction to South African poultry(29, 30). Some of these introduced viruses have persisted in  
328 Egypt since then(31–35). The frequent two-way transmission of AIVs between poultry and wild  
329 birds has provided ample opportunities for the H5N8 HPAI virus to adapt to both poultry and wild  
330 birds through mutation and reassortment, as evidenced by the observed long branch of HA gene  
331 and distinct gene constellation observed in our study. Therefore, the maintenance of HPAI virus  
332 in Egyptian poultry acted as an important source for generating new successful viruses.

333 In addition, Russia-Kazakhstan border is a crucial intersection for various travel routes between  
334 Eastern Europe and China, as well as between European Russia-Western Siberia and Central  
335 Asia(36–38). The wetlands in the vicinity are important breeding grounds or stopping points for

336 birds' migratory across the region(36, 37). Both Kazakhstan and Russia also have a large density  
337 of poultry(38). Since 2014, Russia has recorded H5N8 HPAI outbreaks, initially in Russia's East,  
338 relatively far from Kazakhstan(39). The first recorded outbreak in Kazakhstan occurred in poultry  
339 during the fall of 2020, along the Kazakhstan-Russia border. By year-end, the outbreaks had been  
340 found in eleven provinces of Kazakhstan(37). Migrating birds from Russia may introduce the virus  
341 into Kazakhstan, as earlier outbreaks in Russia have shown(7). Our data also indicate that, between  
342 July and September 2020, the genotype G1 was frequently detected in domestic poultry and  
343 waterfowl near the border between Russia and Kazakhstan. This time and place coincided with the  
344 autumn migration routes of many waterfowl. During the autumn migration period, the wild birds  
345 can travel from their breeding grounds in arctic or temperate breeding sites to the wintering sites  
346 around the world, and disseminate the virus to wider regions, which is known as the primary  
347 mechanism for long-distance transmission of HPAI virus(14). In the 2016/2017 wave, the  
348 previously identified major reassortant AAAAAA8AA also emerged during pre-migratory or early  
349 autumn migration in 2016 somewhere between Belarus and Kazakhstan, and spread westward to  
350 Europe(15), which highlights the ecological significance of the Russian-Kazakhstan border and  
351 autumn bird migration as factors driving viral evolution, and facilitate the spread to Europe.

352 Europe serves as a diversified gene pool for avian influenza viruses due to its location at the  
353 crossroads of various wild bird migration channels connecting Asia, Africa, North America, and  
354 the Arctic. This virus gene pool can provide a large number of genes of wild bird origin to generate  
355 multiple new AIVs, which in turn become the outbreak center of these new viruses(36, 40, 41).  
356 Despite Europe's long history of culling measures to control highly pathogenic avian influenza,  
357 since 2014, HPAI H5 clade 2.3.4.4 viruses have dominated outbreaks in the region, yielding  
358 various subtypes such as H5N1, H5N2, H5N3, H5N4, H5N5, H5N6 and H5N8 through genetic

359 reassortments(14, 15). The majority of HPAI H5 virus detections in wild and domestic birds within  
360 Europe coincide with southwest/westward fall migration and large local water bird aggregations  
361 during wintering(36). This study demonstrates that the H5N8 (G1) virus, which differs from those  
362 (G0) circulating earlier in 2020, was dispersed in the autumn from the Europe-Asia border through  
363 westward migration. This H5N8 virus subsequently spread to at least 19 European countries(36).  
364 Simultaneously, multiple HPAI H5 reassortant viruses were detected in the region(42). The vast  
365 majority of HPAI viruses in wild birds and poultry were H5N8 viruses, while H5N1, H5N3, H5N4,  
366 and H5N5 were mainly isolated from wild birds(20). During this period, a novel H5N1 virus  
367 emerged, which replaced the H5N8 virus and dominated the next wave of 2021/2022. In December  
368 2021, the Europe-origin H5N1 virus was found in Newfoundland, Canada, and then in North  
369 Carolina and South Carolina, USA(43–45). It was suggested that the H5N1 introduction is through  
370 the Atlantic Flyway probably including wild bird migratory routes from northern Europe that  
371 overlap Arctic regions of North America, eventually dispersal farther south into Canada and the  
372 United States(43).

373 The emergence of new dominant genotypes or subtypes for influenza A viruses is usually an  
374 important signal associated with disease outbreaks in animals or humans. In this study, we  
375 observed two dominant replacement events: one is the replacement from G0 to G1 in 2020, and  
376 the other is the replacement from H5N8 to H5N1 in 2021. The two novel HPAI viruses of H5N8  
377 and H5N1 subtype successively caused the most serious 2020/2021 and 2021/2022 outbreaks,  
378 including 11 confirmed human cases, with 7 and 4 described in H5N8 and H5N1 respectively.  
379 Although they both belonged to the clade 2.3.4.4b GS/96 lineage, the H5N8 and H5N1 have  
380 divergent genomes from earlier H5 HPAI viruses. But they shared the same original HA and MP  
381 genes each other. Even when they were widely spread in Europe, Asia, and North America, they

382 later experienced frequent reassortment, their HA and MP genes were always retained. HA gene,  
383 a membrane glycoprotein, mainly influences receptor binding and antigenicity, which makes it  
384 significant in viral pathogenicity, transmission, and cross-species infection(46). MP gene,  
385 encoding M1 and M2 proteins, plays a significant role in the assembly and budding of influenza  
386 virus, determining virus morphology, as well as affecting viral replication, and transmission(47,  
387 48)This suggests that these two segments are critical for viral adaption in wild birds and poultry,  
388 and the acquirements of HA and MP gene may have critical functional effects in breaking host  
389 barriers among wild birds and poultry, leading to worldwide outbreaks. Although other genes may  
390 also have positive effects, such as the N1 gene, further research is needed to clarify its role in viral  
391 adaption.

392 The continuous HPAI outbreaks caused by H5N8 and H5N1 suggested that migratory birds are  
393 not only the natural reservoir, but also potential disease outbreak sources that generate and spread  
394 novel variants. To mitigate the damage caused by bird migration, we face a significant new  
395 challenge. Traditional measures such as killing or immunization are not practical for migratory  
396 birds, and therefore, early warning is the most effective prevention measure.

397 In all, our study on the H5N8 and H5N1 outbreaks emphasizes the importance of identifying risk  
398 factors, such as hot spots and high-risk periods, to control and prevent the global spread of HPAI  
399 viruses in both poultry and wild birds, and even in humans. Comprehensive and systematic  
400 surveillance is needed, with a focus on these risk factors to improve our understanding and  
401 management of HPAI outbreaks.

## 402 MATERIALS AND METHODS

### 403 Data collection

#### 404 Epidemic data

405 Outbreak data were downloaded from FAO Empres-i (<https://empres-i.apps.fao.org>). Number of  
406 cases and death for poultry and wild birds were retrieved from WOAHS six-monthly report  
407 (<https://wahis.woah.org>). Only records of H5Ny HPAI after 2016 were collected, and 7021 records  
408 of Empres-i and 12763 records of WOAHS WAHIS were used. Epidemic wave was defined based  
409 on the epidemic curve of outbreak data. Reported Human cases in H5Ny HPAI data were  
410 summarized from WHO (<https://www.who.int/>).

#### 411 Genetic data

412 A total of 6966 HPAI H5 influenza isolates were retrieved from the Global Initiative on Sharing  
413 All Influenza Data (GISAID) for all available countries and hosts during the period October 2016  
414 to December 2022(49). From this data set, strains with known sampling locations, dates, hosts,  
415 and all segments available were selected. ~~Only one isolate with the earliest sampling date was~~  
416 ~~retained if identical viral genome sequences were found.~~ Sequences that were less than 75% of the  
417 overall length of the segment were also removed. Finally, 3781 strains with unique genomes were  
418 obtained. Among these, 2965 strains with full genome were collected between December 2019 to  
419 December 2022, including 1460 H5N1 strains, 1391 H5N8 strains, which will be refer to as  
420 dataset-N1, dataset-N8 hereafter. All sequence data were aligned using MAFFT v7.310(50) with  
421 default parameters and subsequently manually edited.

422 Given our interest in the genesis of H5Ny viruses, the major agents of 2020-2022 wave, two  
423 expanded dataset ex-dataset-N1 and ex-dataset-N8 were also created. The additional sequences

424 were chosen from Basic Local Alignment Search Tool (BLAST) analysis to be genetically close  
425 to different groups and are not restrict to any specific subtype(51). Groups were defined as a  
426 collection of sequences that are similar to each other in each segment (detailed below). Specifically,  
427 BLAST analysis was run on each group in each segment of dataset-N1 or dataset-N8, collecting  
428 up to 500 sequences from GISAID with a sequence identity no less than 97%. Then, Maximum  
429 Likelihood (ML) phylogenetic trees were estimated using FastTree v2.1.11 with default settings  
430 for all of the unique retrieved sequences and original sequences(52), and sequences within the  
431 larger clade subtending the second ancestor node with bootstrap support no less than 70% of  
432 original sequences were selected. Finally, duplicate sequences were eliminated from these selected  
433 and original sequences. For each included genome, the centroid geographic coordinates of its  
434 sampling location were retrieved at the secondary administrative level using Google Earth  
435 (earth.google.com).

#### 436 **Migration data**

437 Based on the annotation of sampling location of all H5N8 viruses, we have systematically  
438 compiled a list of 108 avian hosts reported to have been infected by HPAI H5N8 viruses (Table  
439 S4). Using the residency data provided by Bow (<https://birdsoftheworld.org>), we have filtered out  
440 78 migratory bird species. By conducting searches on Google Scholar (<https://scholar.google.com>)  
441 using the keywords "host name & migration route", we obtained a total of 228 relevant publications  
442 (those mentioning species migration route information were considered valid). After careful  
443 screening and verification, we excluded literature lacking specific data on migration routes,  
444 resulting in the final confirmation of 29 bird species supported by 48 articles (Fig. S15A, Tables  
445 S4 and S5). To ensure analytical accuracy, we only selected a single data route for each bird species  
446 in different migration directions to avoid bias caused by multiple individuals of the same species

447 sharing the same route data in hotspot analysis. Using the ArcGIS Pro platform, we integrated this  
448 data and employed spatial analysis and data reclassification techniques to extract several important  
449 migration corridors. In order to comprehensively showcase the migration patterns of these 29 host  
450 species, we downloaded corresponding partition vector maps from the IUCN  
451 (<https://www.iucn.org>) website. In ArcGIS Pro, we decomposed the layers of each species based  
452 on breeding and non-breeding periods and overlaid the breeding and non-breeding zones of all  
453 species separately, resulting in an overall distribution map of both breeding and non-breeding areas  
454 for the hosts.

## 455 **Phylogenetic analysis**

### 456 **Maximum likelihood trees**

457 A ML tree for all clade 2.3.4.4b HPAI H5Ny viral HA gene sequences was first constructed using  
458 IQTREE v1.6.12 with “GTR+F+I+G4” model. Node support was determined by 1000 ultrafast  
459 bootstrap replicates. Ancestor time was further determined by TreeTime v0.9.5. Sequence identity  
460 matrix for each gene was calculated using a customized Python v3.10 script and visualized using  
461 heatmap. ML trees were also built for all eight genes for HPAI H5N1 and HPAI H5N8 viruses  
462 isolated between 2020 and 2022 respectively. For each gene segment, only sequence with earliest  
463 sampling date was kept when identical sequences were found. Then, a ML tree was constructed  
464 using IQTREE with same parameters above. These trees were used for further genotypic  
465 assignment. All phylogenetic trees were visualized using a Python package “baltic”.

### 466 **Group and genotype delineating**

467 Within these H5N8 trees, well-supported monophyletic distinct groups were delineated base on  
468 mean paired patristic distance (MPD) (53). For a given internal node, the MPD value is defined as:

469

$$MPD = \frac{\sum d_{ij}}{\binom{n}{2}}$$

470 where  $d_{ij}$  is the phylogenetic distance between sequence  $i$  and  $j$ , and  $n$  is the number of sequences  
471 under this internal node. First, the MPD value for each internal node was calculated. Then, a depth-  
472 first search algorithm was used to find well-supported monophyletic group(54). At each step of  
473 the depth-first visit, a subtree was identified as a distinct group if the MPD value of the subtree  
474 was below a t-percentile threshold of the whole-tree MPD value distribution and the posterior  
475 support of the subtree was no less than 70%. If this condition was met in a node, the search at that  
476 node was stopped, ignoring the children's nodes, passing to analyze other node siblings. The  
477 threshold  $t$  was evaluated and optimized over the range [0th, 100th] percentile of the whole-tree  
478 MPD value distribution, with a step of 0.1. The mean cluster size against the t-percentile was  
479 further plotted. Based on this plot, the last value of  $t$  was chosen as the t-percentile threshold when  
480 the mean cluster size reaches the first plateau, at which value means a relative stable cluster results  
481 with small number of group numbers(55). Above processes were repeated for each segment using  
482 custom script in [https://github.com/DuLab-](https://github.com/DuLab-SYSU/reEmergenceH5Ny/blob/main/tree_processing.ipynb)  
483 [SYSU/reEmergenceH5Ny/blob/main/tree\\_processing.ipynb](https://github.com/DuLab-SYSU/reEmergenceH5Ny/blob/main/tree_processing.ipynb).

484 The index for each monophyletic group was named in alphabet order based on number of  
485 sequences. Hence, the genotype of each strain can be annotated by the group indexes of eight genes  
486 in the order of PB2, PB1, PA, HA, NP, NA, MP, NS, according to a previous study(15). For  
487 example, the genotype aaaaaaaa represents that all the group index of eight gene segments is a.  
488 We also applied the following rules to assign alias for each genotype. Based on the main epidemic  
489 time, genotype G0 and genotype G1 was assigned for viruses if all genes fell into the group  
490 bbbbbbbb or aaaaaaaa, respectively. Within each G0 and G1 series, genotypes were further



491 assigned (for example, G0R1) if any internal gene come from a different monophyletic group. The  
492 decimal number was sequentially assigned based on the number of different genes. Some transient  
493 genotype with only one strain were merged as G0other or G1other based on the group index of  
494 HA gene.

#### 495 **Ancestor status estimation**

496 The extended H5N8 dataset was divided into groups based on the index of monophyletic group  
497 for each segment, resulting in multiple smaller datasets(15). Each group's ancestor status was  
498 estimated using a Bayesian statistical framework. To be more explicit, host status was estimated  
499 using a continuous time Markov chain (CTMC) process and location was estimated using a  
500 Brownian random walk process to model diffusion in continuous space. Despite the fact that  
501 Bayesian framework allows for a joint inference of time, location and host status for ancestor status,  
502 it is computationally challenging for the data set sizes we examined here(56). Because of this and  
503 the fact that both diffusion processes were modelled separately from the substitution process  
504 throughout evolutionary history, the inference problem was divided into two steps: first, only  
505 sequence evolution process was considered to generate an empirical distribution of trees, and then  
506 discrete or continuous trait diffusion processes mentioned above were fitted conditioned on this  
507 set of posterior trees(29, 57). All MCMC sampling analyses were performed using BEAST in  
508 conjunction with the Broad-platform Evolutionary Analysis General Likelihood Evaluator  
509 (BEAGLE) library to enhance computation(58).

510 Bayesian time-resolved phylogenetic trees were first estimated per group per segment using  
511 BEAST v1.10.4(59). Coding genes were partitioned into first + second and third codon positions  
512 for all segments apart from NS and MP segment and applied a separate Hasegawa-Kishino-Yano  
513 85 (HKY85) substitution model with gamma-distributed rate variation among sites to both

514 partitions. An uncorrelated lognormal relaxed molecular clock was used to account for  
515 evolutionary rate variation among lineages and specified a constant population size coalescent tree  
516 prior. Ten independent Markov Monte Carlo (MCMC) chains were run for 200 million generations  
517 and sampled for every 40000 generation with 10% as burn-in. Stationarity and mixing were  
518 investigated using Tracer version 1.5, making sure that effective sample sizes for the continuous  
519 parameters were greater than 200, which is the accepted standard in BEAST analyses. A subset of  
520 500 trees were randomly selected from the combined posterior tree distribution. Then, these trees  
521 were used as an empirical distribution in the subsequent spatial and host diffusion inference. This  
522 is achieved by incorporating a proposal mechanism that randomly draws a new tree from the  
523 empirical distribution. In the discrete host status estimation, the Bayesian stochastic search  
524 variable selection (BSSVS) approach with asymmetric rates was also used to identify best-  
525 supported lineage transitions events between hosts(60). In the continuous geography estimation,  
526 RRW diffusion model was used to perform continuous phylogeographic reconstructions along  
527 groups delineated in the previous step, and a Cauchy distribution was used to model the among-  
528 branch heterogeneity in diffusion velocity(61). Such Bayesian inference resulted in a posterior  
529 distribution of time-measured trees, each annotated with inferred ancestral locations and host status.  
530 The time of MRCA and MRCGA were reported as middle value of posterior distribution with 95%  
531 HPD to quantify the uncertainty. The host of MRCA and MRCGA were reported with the highest  
532 posterior probability. The location of MRCA and MRCGA were reported with the middle value  
533 of posterior distribution for latitude and longitude and using 2-dimension kernel density estimation  
534 to visualization the 95% HPD. The ancestor statuses for specific node were retrieved from  
535 posterior trees using a customized Python script. The trunk host through the time was determined  
536 from posterior phylogenies using PACT v.0.9.5 (<https://github.com/trvr/PACT>). The trunk is

537 comprised of all branches ancestral to a virus that were sampled within a year of the most recent  
538 samples(62).

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554 **AUTHOR CONTRIBUTIONS**

555 J.P., H.S., X.D., J.L., Y.S., G.F.G. and L.L. designed research; J.Z., F.D., L.X., S.S. and Y.L.  
556 contributed to the data collection and processing; J.Z., F.D. and J.P. performed bioinformatics  
557 analyses; J.Z., L.X., F.D. and J.P. performed migration analysis; J.Z. and F.D. wrote the initial  
558 manuscript; J.P., X.D., J.Z., F.D., L.L., J.L., G.F.G., S.J.L., Y.S., L.X., H.S., W.L., J.Z., L.W., S.S.,  
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560 the manuscript.

561 **CODE AVAILABILITY**

562 XML file used for BEAST software and code used for data analysis are available on GitHub at:  
563 <https://github.com/DuLab-SYSU/reEmergenceH5Ny>.

#### 564 **DATA AVAILABILITY**

565 Raw sequence data used in this work are available on GISAID EpiFlu database under strain name  
566 provided in Data S1. Summarized epidemiology data and migration data are available in  
567 Supplemental material.

#### 568 **DECLARATION OF INTERESTS**

569 Authors declare that they have no competing interests.

#### 570 **Supplemental Material**

571 Supplemental figures and tables (Supplemental Material.pdf): **Figures S1 to S16**; Tables S1 to S5.

572 Supplemental data (DataS1.xlsx): Data S1.

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785 **Figure legends**

786 **FIG 1 Epidemiological situation of global HPAI H5Ny during 2016-2022.** (A, F) Epidemic  
787 curve of the confirmed global H5Ny HPAI outbreaks reported to FAO, colored by subtypes (A)  
788 and hosts (F). The temporal span for each epidemic wave is represented by grey square shadows  
789 with black text. (B-E) Counts of confirmed outbreaks in affected countries impacted in four  
790 waves of global HPAI H5Ny epidemics (from yellow for small counts to red color for large  
791 counts). Countries with a grey slash have no outbreaks or statistics. (G) Number of confirmed  
792 cases (filled bar) and deaths (no filled bar) reported to WOAHA in four waves of HPAI H5Ny for  
793 domestic (blue bar) and wild birds (red bar) (in log scale). (H) Reported human cases with circle  
794 size proportion to case number. HPAI stands for high pathogenic avian influenza; FAO stands for  
795 the Food and Agriculture Department of the United Nations; and WOAHA stands for the World  
796 Organization for Animal Health.

797 **FIG 2 Evolution of clade 2.3.4.4b HPAI H5Ny viruses during 2016-2022.** (A) Time-scaled  
798 phylogenetic tree of HPAI clade 2.3.4.4b H5Ny generated using 3781 HA gene sequences. Two  
799 main genetic groups have been denoted and are denoted by arrows in black text. Major epidemic  
800 waves are denoted by grey or white bars, and donations are written in black writing. The  
801 subtypes of each strain colored the tips (red for H5N1, blue for H5N8, gold for H5N6 and grey  
802 for other H5Ny). Isolated regions are depicted on the right column (purple for Europe, light blue  
803 for Asia, pink for North America and green for Africa). (B-I) **Pairwise genetic identity** for each  
804 gene segment of 3781 HPAI H5Ny viruses. Sequences are sorted by the time of isolation. Black  
805 dashed lines separate major epidemic waves and the color from light purple to dark purple  
806 indicates genetic identity from low to high.



807 **FIG 3 Genotype dynamic of HPAI H5N8 viruses during 2020-2021.** (A) Gene constellations  
808 of H5N8 viruses are presented by the order of HA Maximum likelihood tree. Different colors of  
809 tips represent different genotypes. Colored bars on the right show the group classification of  
810 eight gene segments: PB2, PB1, PA, HA, NP, NA, MP and NS. (B) The number of isolated  
811 H5N8 viruses for every half month for different genotypes. (C) The proportion of different  
812 H5N8 viral genotypes. Gaussian kernel density estimation with bandwidth as 0.5 year was used  
813 to calculate the relative frequency at given time points. (D-E) The number of isolated H5N8 by  
814 hosts for G0 H5N8 and G1 H5N8. HPAI: high pathogenic avian influenza; Dom-ans: domestic  
815 *Anseriformes*; Dom-gal: domestic *Galliformes*; Wild-gal: Wild *Galliformes*.

816 **FIG 4 Spread of genotype G0/G1 H5N8 and related migration flyways of wild birds in**  
817 **Eurasia and Africa continents.** (A, C) Continuous spatiotemporal dispersal of genotype G0 and  
818 genotype G1 HPAI H5N8 viruses. The solid lines and dots represent the branches and nodes of  
819 the MCC tree. Contours represent statistical uncertainty of the estimated locations at the internal  
820 nodes (95% HPD based on 2-dimensional kernel density estimates). Dots, lines and contour are  
821 colored according to the time (from red for the earliest to the blue for the latest). (B, D)  
822 Migration pattern summarized from the existing literature. Yellow area represents breeding sites.  
823 Blue represents wintering sites. The purple arrows indicate major G0/G1 H5N8 spread related  
824 southward migration routes. The green arrows indicate major G0/G1 spread related northward  
825 migration routes. The red dots indicate sampling locations of genotype G0 viruses and blue dots  
826 indicate sampling locations of genotype G1 viruses (from light color for the earliest to dark color  
827 for the latest).

828 **FIG 5 Schematics illustrating the generation and spread dynamic of the HPAI H5Ny in**  
829 **2020-2022.** (A) a schematic of key genetic genesis and reassortment history for G0/G1 H5N8

830 and H5N1. Viruses with different colors represent different genotypes or subtypes (gold for G0  
831 H5N8, blue for G1 H5N8, red for H5N1). Orange lines and blue lines represent G0-like and G1-  
832 like genes. Dashed orange lines and dashed blue lines represent non-G0-like and non-G1-like  
833 genes. Red lines represent H5N1-like genes. Grey lines in clouds represent LPAIV pool in  
834 Eurasian and North American waterfowls. Dashed quadrilateral with color from orange to blue  
835 represent dominated genotype replacement from G0 H5N8 to G1 H5N8. **(B)** a schematic of  
836 20/21 H5N8 and 21/22 H5N1 viruses spread between continents. Blue and red dots represent  
837 HPAI H5N8 and HPAI H5N1 outbreaks. Blue rhombuses represent hot spots for gene  
838 source/pool. Red rhombuses represent hot spots for transmission. Blue circles represent G1  
839 H5N8 and red circles represent H5N1, lines in circles represent viral gene segments, and are  
840 colored by gene origin (blue for G1 H5N8, red for H5N1). The number shown in yellow hexagon  
841 indicates the steps of generation and spread of the successive H5N8 and H5N1 panzootic.