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2	Supplementary Information for
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4	Assessing the role of live poultry trade in community-structured transmission of avian
5	influenza in China
6	
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20	This PDF file includes:
21	
22	Supplementary text
23	Figs. S1 to S15
24	Tables S1 to S4
25	Captions for Dataset S1, S2, S3
26	References for SI reference citations
27	
28	Other supplementary materials for this manuscript include the following:
29	
30	Dataset S1, S2, S3 (separate files)
31	Nucleotide alignments, BEAST XML files, and scripts for reconstruction of poultry
32	trade network, community detection, and the following robustness test (available at
33	the GitHub repository: <u>https://github.com/kikiyang/AIVPoultryChina</u>)

34 Supplementary Information Text

35 SI Materials and Methods

36 Sequence data. We accessed haemagglutinin (HA) gene segment sequences of H5N1 37 AIVs sampled from 1996 to 2014 from the GenBank database, and obtained HA gene 38 segment sequences for H7N9 and H5N6 AIVs sampled from 2013 to 2017 from the 39 GISAID database. Duplicate entries and recombinant strains identified using RDP4 (1) 40 were removed. From these sequence sets, we retained only those sampled in China 41 and for which there was known information on date and location of sampling. In order 42 to focus on virus dissemination among poultry, it was first necessary to exclude from 43 the viral phylogenies those chains of AIV transmission that derive from interspecies 44 transmission events and which occur in wild bird populations. This was achieved by 45 reconstructing the movement of AIV lineages between wild birds and domestic 46 poultry using a range of sampling and analysis strategies. AIV sequences that belong 47 to phylogenetic clusters that are inferred to represent transmission wholly or 48 predominately in or from wild birds were removed from the data sets (SI Appendix, 49 Fig. S7), which means that poultry AIV sequences found within clades dominated by 50 wild bird AIV sequences were also excluded. Consequently, only those lineages that 51 are inferred to be circulating in domestic poultry population were retained for further 52 analysis.

53

54	In order to ameliorate potential sampling biases, we subsequently randomly
55	subsampled these datasets in a stratified manner to create a more equitable
56	spatiotemporal distribution of AIV sequences. Specifically, the H5N1 HA gene
57	sequences were subsampled to 322 sequences (1704 nt); sequences from
58	over-sampled years and provinces were removed randomly so that there was at most 8
59	sequences per year and per province, thereby increasing the evenness of sampling
60	whilst retaining a wide range of sampling dates and locations (SI Appendix, Fig. S11).
61	In addition, those locations for which there are too few (<4) H5N1 sequences to be
62	analysed in phylogeographic dynamics were removed. Similarly, HA sequences of
63	H7N9 and H5N6 viruses were subsampled to 291 sequences (444 sequences for the
64	full-host dataset of H7N9) and 201 sequences, respectively (SI Appendix, Fig. S11).
65	Accession numbers of all sequences in the final datasets are provided in Dataset S2.
66	
67	Initial maximum likelihood (ML) phylogenies were estimated for each subtype using
68	FastTree v2.1.4 (2) under a GTR+ Γ nucleotide substitution model. These phylogenies
69	were then used to test for the presence of phylogenetic temporal structure, by
70	generation of a scatterplot of root-to-tip genetic divergence against date of sampling
71	using TempEst v1.5 (3). Strong phylogenetic temporal structure was detected in all
72	datasets (SI Appendix, Fig. S12).

74	Phylogeographic inference. Time-resolved phylogenies of HA sequences were
75	estimated using the Markov chain Monte Carlo (MCMC) approach implemented in
76	BEAST v1.8.2 (4) with the BEAGLE (5) library. We used a uncorrelated lognormal
77	(UCLN) relaxed molecular clock model (6), the SRD06 nucleotide substitution model
78	(7) and the Gaussian Markov random field (GMRF) Bayesian Skyride coalescent tree
79	prior (8). For each dataset, MCMC chains were run in triplicate for 100 million
80	generations with burn-in of 10%, sampling every 10,000 steps. Convergence of
81	MCMC chains was checked with Tracer v1.7. A set of 1000 trees was subsampled
82	from the MCMC chain and used as an empirical tree distribution for the subsequent
83	analysis.
84	
85	Time-measured phylogenies were inferred using the Bayesian discrete
86	phylogeographic approach (9) implemented in BEAST v1.8.2 (4). We used a
87	non-reversible discrete-state continuous time Markov chain (CTMC) model and a
88	Bayesian stochastic search variable selection (BSSVS) approach to infer (i) the most
89	probable locations of ancestral nodes in the phylogeny and (ii) the history and rates of
90	lineage movement among locations (9). For each dataset, one MCMC chain was run
91	for 200 million steps with a burn-in of 10% steps, sampled every 20,000 steps.
92	Similarly, we assessed the convergence of the chains in Tracer v1.7. Using
93	TreeAnnotator v1.8.2, we subsequently summarized a maximum clade credibility tree
94	from the posterior set of trees of each dataset. To ensure that the inferred relationships

95 between distance and lineage movement were not a consequence of the prior used, we
96 repeated the analysis after randomising the locations assigned to each sequence (*SI*97 *Appendix*, Fig. S13).

99	Quantifying contributions of potential predictors of AIV dispersal. To infer
100	potential explanatory factors that are associated with AIV dispersal among poultry in
101	different locations, we applied the generalized linear model (GLM) extension of
102	Bayesian phylogeographic inference (10) to the HA gene datasets of H5N1, H5N6
103	and H7N9 viruses in China. We used a Bayesian Stochastic Search Variable Selection
104	(BSSVS) approach with binomial prior probability distributions on the indicator
105	variables reflecting a 50% prior probability on no predictors being included. We used
106	Bayes factors (BFs) to quantify the support for the posterior probability of inclusion
107	of each potential predictive factor.
108	
109	The potential predictors used in the model were (i) among-province poultry trade flux,
110	obtained using from the reconstructed live poultry trade network (outlined above), (ii)
111	egg trade flux among provinces in the reconstructed egg trade network (outlined
112	above), (iii) the shortest distance among provinces along the national highway
113	network (see above), (iv) socio-economic predictors relating to production and
114	consumption of live poultry and poultry egg (described in SI Appendix, Table S4),
115	specifically, poultry population density, poultry production, poultry consumption and

116	per capita consumption, egg production, egg consumption and per capita
117	consumption, and demographic data, (v) absolute latitude and longitude of the capital
118	city of each province, (vi) number of sequences from each location, and (vii) the
119	existence of bird migration among provinces. Bird migration flux was ascertained
120	from a binary network of wild bird migration among provinces, based on GPS
121	tracking of wild bird migration routes in China from 2006 to 2016, obtained from the
122	Chinese Academy of Forestry (11). All the data (Dataset S3) were log-transformed
123	and normalized as the model inputs.
124	
125	Interspecies transmission inference. The host transmission between wild avian hosts
126	and domestic poultry hosts throughout the viral evolutionary history was inferred
127	using a Bayesian discrete phylogeographic approach (9) implemented in BEAST
128	v1.8.2 (4). We implemented a non-reversible discrete-state continuous time Markov
129	chain (CTMC) model and a Bayesian stochastic search variable selection (BSSVS)
130	approach to infer the most probable ancestral host of the phylogeny and the most
131	parsimonious description of the phylogeography dynamics (9). MCMC chains were
132	run for 200 million steps with a burn-in of 10% steps, and sampled every 20,000 steps.
133	The maximum clade credibility tree was summarized by TreeAnnotator v1.8.2. Three
134	datasets with different sampling strategies are used in the inference. The host species
135	of each AIV gene sequence is defined according to the United States Geological

136	Survey (USGS) (12) and by information in the original references. All three trees
137	have a backbone of domestic poultry states (Supplementary Fig. 5).
138	
139	Sampling strategies. Dataset 1: 175 sequences of domestic poultry and 142
140	sequences of wild birds. We subsampled the HA gene segments of H5N1 viruses from
141	domestic poultry to 175 sequences (1704 nt), at most 2 sequences per year and per
142	province so that the amount of sequences of domestic poultry is nearly equal to that of
143	wild birds (142 sequences). Sequences of wild birds are retained as 142 sequences
144	without down-sampling.
145	
146	Dataset 2: 353 sequences of domestic poultry and 142 of wild birds. We subsampled
147	the HA gene segments of H5N1 viruses from domestic poultry to 353 sequences
148	(1704 nt), at most 8 sequences per year and per province. Sequences of wild birds are
149	retained as 142 sequences without down-sampling.
150	
151	Dataset 3: 285 HA sequences of H5N1 of domestic poultry and 84 of wild birds.
152	Sequences of domestic poultry and wild birds are together randomly subsampled to 5
153	sequences per year, per host type, per location.
154	
155	Poultry transportation network reconstruction. Provincial-level networks of
156	poultry transportation (Fig. 3, SI Appendix, Fig. S6) were constructed from statistics

157	of poultry egg production and populations of domestic poultry, using a classic gravity
158	model. This model is supported by cross-sectional surveys of poultry transportation in
159	Cambodia (13) and Vietnam (14). In summary, the flux of live poultry or poultry egg
160	transport (G_{ij}) between provinces <i>i</i> and <i>j</i> separated by geographic distance d_{ij} takes the
161	form $G_{ij} = N_i N_j d_{ij}^{-1}$, where N_i is the amount of live poultry (unit: 10,000 poultry) or
162	poultry egg production (unit: ton) in source province i (averaged across years). N_j is
163	human population size (unit: 10,000 people) in destination province <i>j</i> (averaged across
164	years). Both N_i and N_j were obtained from statistical yearbooks and databases (SI
165	Appendix, Table S4); d_{ij} is the shortest distance (unit: kilometre) between provinces in
166	the national highway network, calculated by the Origin-Destination Cost Matrix
167	algorithm in ArcGIS v10.2 (ESRI, Redlands, CA, USA). This distance was chosen
168	because inter-provincial live poultry transportation in China mainly occurs via
169	national highways; live poultry transportation on freeways is limited due to high tolls,
170	and on railways due to regulations (15, 16). Additionally, other gravity-model
171	parameterisations were tested and the results showed that the network structure we
172	infer is robust to the parameterisation.
173	

174 **Parameter estimation of gravity model.** A model with an exponential distance

- 175 decay function and parameters fit to a dataset from Cambodia (13, 17) was calculated
- as a contrast to the live poultry trade network. The framework is as follows:
- 177 $G_{ij} = \theta N_i^{\varepsilon} N_j^{\beta} f\left(d_{ij}\right)$

178
$$f(d_{ij}) = e^{-\frac{d_{ij}}{\lambda}} / \lambda^2$$

where $\lambda = 167.456$, $\varepsilon = 0.543$, and $\beta = 0.934$ are fitted parameters with data from a cross-sectional survey on live poultry traders in Cambodia, and θ is a scaling parameter equalling 1. The correlation coefficient between trade flows estimated from this model and from the model with classic parameters is 0.698.

183

184 Hidden viral transmission path detection. To describe the process of virus 185 transmission across all provinces, including those for which no AIV sequence data 186 was present in our data sets, we used a previously-published gene flow network model 187 (GFN) (18) to infer an empirically-derived weighted network of AIV dispersal (SI Appendix, Fig. S5). The GFN model is based on the observation that the pairwise 188 189 genetic distance between a pair of AIV sequences from two different locations is 190 strongly correlated with the duration of the transmission history between the two 191 infections, which itself is a consequence of the path taken by that history through a 192 given network of locations, whose connections are weighted by some measure (e.g. 193 distance, or trade). A summary of the steps of the GFN model approach follows here. 194 (i) To begin we construct a fully-adjacent network, whose nodes are the geographic 195 locations/provinces (regardless of whether AIV sequences are available for that 196 location) and whose edges represent the minimum distance along national highways 197 between adjacent locations (edges between non-adjacent locations are not excluded). 198 (ii) A hypothesised path through this fully-adjacent network is then proposed. The

199	path must pass through all locations for which virus sequences are available in a
200	sequential random order; in doing so, the path may pass through locations without
201	virus sampling and may visit the same node more than once. The shortest path
202	meeting these conditions was identified using the Floyd-Warshell Algorithm (19). (iii)
203	A pair of sequences from two locations is chosen. (iv) The spatial distance along the
204	hypothesised path between the locations defined by these two sequences is then
205	calculated. This is simply the sum of the edges in the fully-adjacent network as the
206	path moves through it. (v) Steps (ii)-(iv) are then repeated for all pairs of sequences.
207	The correlation between the genetic distance and path spatial distance for each pair of
208	sequences is then computed. (vi) Steps (ii) to (v) are then repeated 10,000 times,
209	resulting in a distribution of correlation coefficient values. (vi) The top 10% of these
210	replicates with the highest correlation coefficients are identified. (vii) The
211	hypothesised paths that correspond to the top 1000 paths are then summarised into the
212	final gene flow network (GFN). This is done by simply counting the frequency with
213	which each edge in the fully-adjacent network is represented in the set of 1000 paths.
214	These frequencies then reflect the likelihood of virus movement among all locations,
215	including via locations for which no sequences were sampled.
216	
217	Community structure detection. Identifying community structures (20) is a crucial

218 step in investigating networks that might explain patterns of viral spatial

219 dissemination. In this context, a "community" is a group of nodes in a network such

220	that intra-group connections are stronger or more numerous than inter-group
221	connections (21, 22); the degree to which a network is subdivided into communities is
222	measured as "modularity" (22). Using the Walktrap community finding algorithm
223	(23), with random walk length $t = 5$, we identified the community structure of (i) the
224	live poultry trade networks, (ii) the poultry egg trade network. Although each of these
225	networks are directed, edge directions were ignored in order to focus on vertex
226	connections. The connection strength of two vertices was defined as the sum of the
227	weights of all edges between those vertices; this approach has proven suitable for
228	modularity-based community detection algorithms (20, 22).
229	
230	To set the random walk length t to a suitable value, we experimented the community
231	structure detection with t from 1 to 100. We observed that small values like $t=1, 2, 3$,
232	4, 5 generated community structures with much higher modularity, which is
233	consistent with the previous study (24). When $t=2$, the modularity value is highest,
234	whereas locations are merged into only two large communities, which probably
235	results from the resolution limit of modularity optimization (25). To sufficiently
236	capture the local community structure and avoid over optimization of modularity as
237	well, we set the random walk length <i>t</i> to 5.
238	
239	To test the robustness of the detected community structures, we introduced the
240	random perturbation as Gaussian noise (mean=0, standard deviation=10% of the mean

242	the community structure detection by Walktrap community finding algorithm, with			
243	random walk length $t=5$. To quantify differences in community structure of the			
244	perturbed network and that of the original network, we used four representative			
245	methods including the Rand index (26), van Dongen metric (27), normalized mutual			
246	information (28) and variation of information (29). The results (Dataset S1) show that			
247	the detected community structures are stable.			
248				
249	References			
250	1. D. P. Martin, B. Murrell, M. Golden, A. Khoosal, B. Muhire, RDP4: Det	ection		
251	and analysis of recombination patterns in virus genomes. Virus Evol 1, v	ev003		
252	(2015).			
253	2. M. N. Price, P. S. Dehal, A. P. Arkin, FastTree: computing large minimu	ım		
254	evolution trees with profiles instead of a distance matrix. Mol Biol Evol 2	26,		
255	1(41, 1(50, (2000)			

edge weights) into the live poultry network. On the perturbed networks, we conducted

255 1641-1650 (2009).

- A. Rambaut, T. T. Lam, L. M. Carvalho, O. G. Pybus, Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen).
 Virus Evol 2, vew007 (2016).
- 4. A. J. Drummond, M. A. Suchard, D. Xie, A. Rambaut, Bayesian phylogenetics
 with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29, 1969-1973 (2012).
- 5. D. L. Ayres *et al.*, BEAGLE: an application programming interface and
 high-performance computing library for statistical phylogenetics. *Syst Biol*,
 syr100 (2011).
- A. J. Drummond, S. Y. Ho, M. J. Phillips, A. Rambaut, Relaxed phylogenetics
 and dating with confidence. *PLoS Biol* 4, e88 (2006).
- 266 7. B. Shapiro, A. Rambaut, A. J. Drummond, Choosing Appropriate Substitution
 267 Models for the Phylogenetic Analysis of Protein-Coding Sequences. *Mol Biol*268 *Evol* 23, 7-9 (2005).
- V. N. Minin, E. W. Bloomquist, M. A. Suchard, Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. *Mol Biol Evol* 25, 1459-1471 (2008).
- 9. P. Lemey, A. Rambaut, A. J. Drummond, M. A. Suchard, Bayesian
- 273 phylogeography finds its roots. *PLoS Comput Biol* **5**, e1000520 (2009).

274	10.	P. Lemey et al., Unifying viral genetics and human transportation data to
275		predict the global transmission dynamics of human influenza H3N2. PLoS
276		Pathog 10, e1003932 (2014).
277	11.	S. Li et al., Migratory Whooper Swans Cygnus cygnus Transmit H5N1 Virus
278		between China and Mongolia: Combination Evidence from Satellite Tracking
279		and Phylogenetics Analysis. Sci Rep 10.1038/s41598-018-25291-1 (2018).
280	12.	United States Geological Survey, List of Species Affected by H5N1 (Avian
281		Influenza). United States Geological Survey. Available at
282		https://www.nwhc.usgs.gov/disease_information/avian_influenza/affected_spe
283		<u>cies_chart.jsp</u> . Deposited 2011.
284	13.	M. D. Van Kerkhove, H5N1/highly pathogenic avian influenza in Cambodia :
285		evaluating poultry movement and the extent of interaction between poultry
286		and humans. PhD thesis, London School of Hygiene & Tropical Medicine.
287		http://dx.doi.org/10.17037/PUBS.00682389 (2009).
288	14.	G. Fournié et al., Investigating poultry trade patterns to guide avian influenza
289		surveillance and control: a case study in Vietnam. Sci Rep 6, 29463 (2016).
290	15.	K. Bingsheng, H. Yijun, "Poultry sector in China: structural changes during
291		the past decade and future trends" in Poultry in the 21st Century: avian
292		influenza and beyond. Proceedings of the International Poultry Conference,
293		held 5–7 November 2007, Bangkok, Thailand., O. Thieme, D. Pilling, Eds.
294		(FAO, Rome, 2008), pp. 85-117.
295	16.	L. Fang et al., Environmental factors contributing to the spread of H5N1 avian
296		influenza in mainland China. PLoS One 3, e2268 (2008).
297	17.	M. D. Van Kerkhove, "Poultry Movement and Sustained HPAI Risk in
298		Cambodia" in Health and animal agriculture in developing countries, D.
299		Zilberman, J. Otte, D. Roland-Holst, D. Pfeiffer, Eds. (Springer, 2011),
300		https://doi.org/10.1007/978-1-4419-7077-0, pp. 233-263.
301	18.	H. Tian et al., Avian influenza H5N1 viral and bird migration networks in
302		Asia. Proc Natl Acad Sci USA, doi: 10.1073/pnas.1405216112 (2014).
303	19.	R. W. Floyd, Algorithm 97: shortest path. Commun ACM 5, 345 (1962).
304	20.	M. Girvan, M. E. Newman, Community structure in social and biological
305		networks. Proc Natl Acad Sci USA 99, 7821-7826 (2002).
306	21.	F. Radicchi, C. Castellano, F. Cecconi, V. Loreto, D. Parisi, Defining and
307		identifying communities in networks. Proc Natl Acad Sci USA 101, 2658-2663
308		(2004).
309	22.	M. E. Newman, M. Girvan, Finding and evaluating community structure in
310		networks. Phys Rev E 69, 026113 (2004).
311	23.	P. Pons, M. Latapy, Computing communities in large networks using random
312		walks. J. Graph Algorithms Appl. 10, 191-218 (2006).
313	24.	D. Lai, H. Lu, C. Nardini, Enhanced modularity-based community detection
314		by random walk network preprocessing. Phys Rev E 81, 066118 (2010).

315 25. S. Fortunato, M. Barthélemy, Resolution limit in community detection. Proc 316 Natl Acad Sci USA 104, 36-41 (2007). 317 L. Hubert, P. Arabie, Comparing partitions. J Classif 2, 193-218 (1985). 26. S. van Dongen, Performance criteria for graph clustering and Markov cluster 318 27. 319 experiments. Technical Report INS-R0012, National Research Institute for 320 Mathematics and Computer Science in the Netherlands, Amsterdam (2000). 321 28. D. Leon, D.-G. Albert, D. Jordi, A. Alex, Comparing community structure identification. J Stat Mech 2005, P09008 (2005). 322 323 29. M. Meilă, Comparing clusterings—an information based distance. J Multivar 324 Anal 98, 873-895 (2007). 325



- 327 Fig. S1. The maximum clade credibility (MCC) phylogeny of the HA gene of
- 328 H5N1 (A), H5N6 (B) and H7N9 (C) viruses in poultry in China. The phylogeny is
- 329 inferred by Bayesian phylogeography inference methods. Branches are coloured
- according to the most probable location state of their descendent nodes.
- 331



333	Fig. S2. Contributions of all predictor variables to the dissemination of H5N1,
334	H5N6 and H7N9 lineages in China among poultry, inferred from analysis of HA
335	gene sequences. HP AIV subtypes H5N1, H5N6 and H7N9 are coloured blue, green
336	and red, respectively. Predictors labelled (O) and (D) represent the origin and
337	destination, respectively. In the left-hand plots, the estimated coefficients of
338	predictors are represented as circles (>0 = positive association, <0 = negative
339	association). Error bars represent the 95% highest posterior density (HPD) credible
340	interval for these estimates. The bars in the right-hand plots show the posterior
341	probability of inclusion of each predictor.



345 Fig. S3. Contributions of predictor variables to the dissemination of H5N1, H5N6

346 and H7N9 lineages among poultry in China, inferred from analysis of HA gene

347 sequences, without minimum road network distance. Results when the minimum

- 348 road network distance predictor is excluded from the analysis. Due to the high
- similarity of poultry trade and egg trade (R=0.95, P<0.01), we do not further
- 350 differentiate between those two predictors.
- 351



- 353 Fig. S4a. Sensitivity analysis, H5N1 AIV. Results show the effect of the inclusion of
- 354 the third predictor on the estimates of wild bird migration and live poultry trade
- 355 network.
- 356



- 358 Fig. S4b. Sensitivity analysis, H5N6 AIV. Results show the effect of the inclusion of
- the third predictor on the estimates of wild bird migration and live poultry trade
- 360 network.
- 361
- 362



- Fig. S4c. Sensitivity analysis, H7N9 AIV. Results show the effect of the inclusion of
 the third predictor on the estimates of wild bird migration and live poultry trade
- network. Due to the high similarity of poultry trade and egg trade (R=0.95, P<0.01),
- 367 we do not further differentiate between those two predictors.
- 368
- 369



Fig. S5. The gene flow network model. (A) Method of finding the virus transmission 371 372 path (grey arrow-headed solid lines) in time order (t): a - b - e - b - e - c - f - g - dthat is hidden in the full adjacent network under the observed path (blue arrow-headed 373 374 dotted lines): a->b->c->d. Blue nodes indicate locations from which virus sequences are available; grey indicates locations without virus sampling. Black solid lines 375 between nodes represent edges between adjacent locations in the full adjacent 376 network. Line widths represent edge weights that are used to find the shortest path by 377 378 Flovd-Warshell algorithm. (B) Histogram of the correlation coefficients of the path 379 distances through the network and the gene flow network of sequences. Bars represent 380 the correlation coefficients generated by 10,000 hypothesised transmission paths and 381 genetic distances, 1000 best-fittings were highlighted in black. The blue dotted line indicates the strength of the correlation obtained if the distance used is simply the 382 383 geodesic distance between pairs of provinces. (C) Gene flow network. Frequency of migration events between pairs of provinces in 1000 top-selected randomly generated 384 385 transmission paths. Green and dark blue curves represent low and high frequencies, 386 respectively. Blue indicates provinces from which virus sequences are available; grey 387 indicates provinces without virus sampling.



- **Fig. S6. Poultry egg trade network and community structure.** Upper panel:
- 391 Accessibility of poultry egg trade flows between pairs of provinces. Colours of light
- 392 purple and dark purple represent low and high accessibility respectively. Lower panel:
- 393 poultry egg trade communities 1, 2, 3 (coloured pink, purple, and yellow, respectively)
- 394 are clustered in the Yangtze River Delta region, north-eastern China and other regions.



396	Fig. S7a. The maximum clade credibility (MCC) phylogeny of the HA gene of
397	H5N1 viruses in wild birds and domestic poultry in China inferred by Bayesian
398	discrete phylogeographic approach. Light blue and light red represent the host type
399	of wild birds and domestic poultry, respectively. The phylogeny is inferred on Dataset
400	<i>l</i> (sequences of domestic poultry: 175 and those of wild birds: 142). The sequences
401	indicated by a black rectangle are in the wild bird lineage that would be removed in
402	subsequent analysis.
403	



407 Fig. S/b. The maximum clade credibility (MCC) phylogeny of the HA gene of	407	Fig. S7b. The maximum clade credibility (MCC) phylogeny of the HA gene of
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408 H5N1 viruses in wild birds and domestic poultry in China inferred by Bayesian

409 **discrete phylogeographic approach.** Light blue and light red represent the host type

410 of wild birds and domestic poultry, respectively. The phylogeny is inferred on Dataset

411 2 (sequences of domestic poultry: 353 and those of wild birds: 142). The sequences

- 412 indicated by a black rectangle are in the wild bird lineage that would be removed in
- 413 subsequent analysis.
- 414
- 415



417	Fig. S7c.	The maximum	clade credibility	y (MCC) pl	hylogeny of th	e HA gene of
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418 H5N1 viruses in wild birds and domestic poultry in China inferred by Bayesian

419 **discrete phylogeographic approach.** Light blue and light red represent the host type

420 of wild birds and domestic poultry, respectively. The phylogeny is inferred on Dataset

421 3 (sequences of domestic poultry: 285 and those of wild birds: 84). The sequences

422 indicated by a black rectangle are in the wild bird lineage that would be removed in

- 423 subsequent analysis.
- 424



Fig. S8. Community detection at different scales. (A) Multi-scale partitions of the
live poultry trade network found by Walktrap algorithm, and (B) the corresponding
modularity value at various resolution levels. The triangle indicates the live poultry
trade communities (Partition 4) used in the main text. Four community structures at
different scales (dotted lines) were selected for comparison. Positive value of the
modularity indicates the possible presence of community structure.





435 (Partition 3, 5, and 9). The raw transition rates between locations are obtained from

436 the analyses reported in Fig. 1. Two means are shown here: blue = mean transition

437 rate for pairs of locations in different live poultry trade communities (LPTCs); red =

438 mean transition rate for pairs of locations in the same LPTC. Dotted vertical lines

439 show these two means calculated using the partition of the empirically-derived ("true")

- 440 LPTC network.
- 441



443 Fig. S10. Histograms of mean among-location AIV lineage transition rates

444 (Partition 1 and 15). The raw transition rates between locations are obtained from the

analyses reported in Fig. 1. Two means are shown here: blue = mean transition rate

446 for pairs of locations in different live poultry trade communities (LPTCs); red = mean

transition rate for pairs of locations in the same LPTC. Dotted vertical lines show

these two means calculated using the partition of the empirically-derived ("true")

- 449 LPTC network.
- 450



452 Fig. S11a. Spatial and Temporal distribution of HA sequences of H5N1 viruses

453 used in this study before/after down-sampling. Upper panel: Spatial distribution of

454 HA sequences from domestic poultry between provinces in China after

down-sampling. Pie size represents the number of sequences. Lower panel: Temporal

distribution of HA sequences from domestic poultry from 1996 to 2017. The dark bar

457 represents sequences before down-sampling and the light bars represent

458 down-sampled sequences.



460 Fig. S11b. Spatial and Temporal distribution of HA sequences of H5N6 viruses

461 used in this study before/after down-sampling. Upper panel: Spatial distribution of

462 HA sequences from domestic poultry between provinces in China after

down-sampling. Pie size represents the number of sequences. Lower panel: Temporal

464 distribution of HA sequences from domestic poultry from 2013 to 2018. The dark bar

465 represents sequences before down-sampling and the light bars represent

466 down-sampled sequences.



468 Fig. S11c. Spatial and Temporal distribution of HA sequences of H7N9 viruses

469 used in this study before/after down-sampling. Upper panel: Spatial distribution of

470 HA sequences from domestic poultry between provinces in China after

down-sampling. Pie size represents the number of sequences. Lower panel: Temporal

distribution of HA sequences from domestic poultry from 2013 to 2017. The dark bar

473 represents sequences before down-sampling and the light bars represent

474 down-sampled sequences.





476 Fig. S12. Strong temporal signal tested in TempEst of H5N1 (A), H5N6 (B) and

477 H7N9 (C). We used the subsampled dataset of sequences of the HA gene segments of

478 each subtype of viruses isolated from domestic poultry in China in this test.



- 480 Fig. S13. No associated between lineage migration rates and distances when
- 481 sequence locations of AIV subtypes H5N1, H5N6 and H7N9 are randomized
- 482 before phylogeographic analysis using the same method and datasets used in
- 483 **Figure 1.** (A) H5N1 (R = -0.052, P = 0.765); (B) H5N6 (R = -0.096, P = 0.566); and
- 484 (C) H7N9 (R = -0.096, P = 0.566).
- 485



487 Fig. S14. Community detection using Walktrap, Fast Greedy, Louvain,

488 **modularity optimization.** The hierarchical structure in the left panel is identified

489 using the Walktrap algorithm. Location are coloured according to the communities

490 detected by Walktrap (left column), and by fast greedy, Louvain and modularity

491 optimization (right column).





Fig. S15. Histograms of mean AIV lineage transition rates among-location with
communities detected by the Fast Greedy, Louvain, modularity optimization.

494 communities detected by the Fast Greedy, Louvain, modularity optimization.

The raw transition rates between locations are obtained from the analyses reported in Fig. 1. Two means are shown here: blue = mean transition rate for pairs of locations

496 Fig. 1. Two means are shown here: blue = mean transition rate for pairs of locations
497 in different communities; red = mean transition rate for pairs of locations in the same

498 community. Dotted vertical lines show these two means calculated using the partition

499 of the empirically-derived ("true") LPTC network.

Predictor	Inclusion*	BF [†]	cEffect (95% HPD) [‡]
H5N1			
Viral sample size (D)	1	infinite	0.588 (0.370,0.811)
Poultry trade	0.193	9	1.200 (0.678, 1.772)
Bird migration	0.807	149	1.478 (0.925, 2.007)
H5N6			
Viral sample size (D)	1	infinite	0.776 (0.442, 1.138)
Poultry trade	0.814	156	0.630 (0.128, 1.194)
Bird migration	0.186	8	0.621 (-0.239, 1.413)
H7N9			
Viral sample size (D)	1	infinite	0.871 (0.581, 1.176)
Poultry trade	0.912	367	1.159 (0.315, 2.020)
Bird migration	0.088	3	0.669 (-0.257, 1.477)

Table S1. Generalized linear model results (controlling for viral sample size)

*Inclusion, probability that the predictor was included in the model. *BF, Bayes factor.

[‡]cEffect, conditional effect size, which represents the estimate of the coefficient conditional on the predictor being included in the model. Both the mean and the 95% highest posterior density credible interval (95% HPD) of the conditional effect size are reported.

 Table S2. Out-degree and in-degree of province nodes in live poultry trade network.

	out-degree	in-degree
Beijing	0.0066691	0.0170491
Tianjin	0.0046496	0.0104736
Hebei	0.1070538	0.0637392
Shanxi	0.0163548	0.0382506
Neimenggu	0.007919	0.0149981
Liaoning	0.0389668	0.0246289
Jilin	0.0199215	0.0170071
Heilongjiang	0.0147619	0.0199825
Shanghai	0.0044865	0.0173906
Jiangsu	0.077618	0.0774893
Zhejiang	0.0236046	0.0443643
Anhui	0.0605377	0.067238
Fujian	0.0146695	0.0218523
Jiangxi	0.0398436	0.0369054
Shandong	0.1180784	0.0743098
Henan	0.1252846	0.0848688
Hubei	0.0621741	0.0543692
Hunan	0.0509017	0.0538547
Guangdong	0.0516537	0.0516608
Guangxi	0.0336637	0.0258277
Hainan	0.0055096	0.0042646
Chongqing	0.0171049	0.0215302
Sichuan	0.0530139	0.0454635
Guizhou	0.0111807	0.0244125
Yunnan	0.0136839	0.0222702
Tibet	7.65E-05	0.0007838
Shannxi	0.0101696	0.0319031
Gansu	0.00568	0.0151643
Qinghai	0.0003532	0.0028651
Ningxia	0.0015283	0.0036118
Xinjiang	0.002401	0.00497
Hong_Kong	0.0004851	0.0065007

	out-degree	in-degree
Hebei	0.046641746	0.046641745
Shanxi	0.013095849	0.013095849
Liaoning	0.046641746	0.046641746
Jilin	0.023406387	0.023235359
Jiangsu	0.034449901	0.033961251
Zhejiang	0.028097438	0.028390628
Anhui	0.050550955	0.050550955
Fujian	0.023504117	0.023650713
Jiangxi	0.040875663	0.040875663
Shandong	0.032959515	0.033350437
Henan	0.072784578	0.072637981
Hubei	0.085074153	0.085171883
Hunan	0.092135161	0.092232891
Guangdong	0.072295928	0.0721249
Guangxi	0.04439395	0.044540547
Chongqing	0.026069535	0.026069535
Sichuan	0.041217719	0.041119988
Guizhou	0.047570183	0.047570182
Yunnan	0.022502382	0.02247795
Shaanxi	0.063133719	0.06313372
Gansu	0.045933201	0.045933201
Xinjiang	0.022942168	0.022991033
Hong_Kong	0.02372401	0.023601847

Table S3. Out-degree and in-degree of province nodes in gene flow network.

Indicator	Mainland China (except HK)	Source	НК	Source
Poultry population	RESID ^a	EPS ^b	Maximum Rearing Capacity	ACFD ^c
Poultry production	Slaughtered poultry	NBS ^d	Estimated Quantities of Chickens, ducks and quails	ACFD
Poultry egg production	Output of poultry eggs	NBS	Estimated Quantities of Hatching Hen Eggs and Table Hen Eggs	ACFD
Poultry meat production	Output of poultry meat	EPS	Local production of live poultry	ACFD
Human population	Total population	EPS	Total Population	World Bank Open Data
Poultry egg consumption per capita	Per capita consumption expenditure of rural households, poultry egg	EPS	Poultry egg consumption / Human population	Calculation of statistics
Poultry egg consumption	Poultry egg consumption per capita × Human population	Calculation of statistics	Eggs of wholesale markets throughput	ACFD
Poultry consumption per capita	Per capita consumption expenditure of rural households, poultry	EPS	Consumption of live poultry / Human population	Calculation of statistics
Poultry consumption	Poultry consumption per capita × Human population	Calculation of statistics	Consumption of live poultry	ACFD

Table S4. Indicators and data sources of statistics.

a. RESID is the abbreviation of number of individuals existing at the end of the calendar year(residual poultry);

b. Database: EPS China data (http://www.epschinadata.com/) offering time serial statistical data (including census data) from statistical yearbooks;

c. Agriculture, Fisheries and Conservation Department, The Government of the Hong Kong Special Administrative Region;

d. National Bureau of Statistics;

Additional Dataset S1 (separate file)

Permutation of community structure and comparison.

Additional Dataset S2 (separate file)

Accession number of all sequences in this study.

Additional Dataset S3 (separate file)

Original data of predictors for GLM.