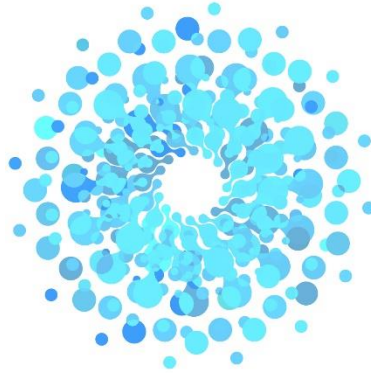


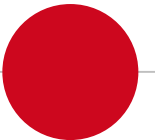


The 2024-2025 clade 2.3.4.4b epidemic wave - a genetic overview -

Alice Fusaro

Istituto Zooprofilattico Sperimentale delle Venezie (IZSve), Padua, Italy

16th October 2025





Genetic diversity, 2020-2025

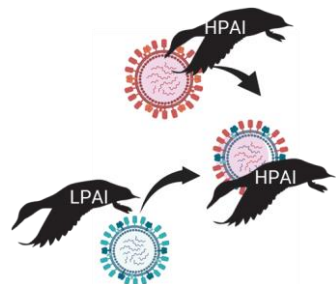
W1 - 2020-2021									
	PB2	PB1	PA	HA	NP	NA	MP	NS	
A	20	20	20	20	20	20	20	20	
B	35	28	1	20	1	30	20	20	
C	1	1	1	20	1	1	20	1	
D	20	20	20	20	20	27	20	20	
E	20	20	29	20	20	27	20	20	
F	27	20	29	20	37	27	20	20	
G	31	31	32	20	12	17	20	1	
H	20	31	29	20	27	63	20	20	
I	25	14	25	20	26	13	20	28	
J	31	23	5	20	26	2	20	28	
L	25	1	1	20	1	13	20	1	
M	20	20	14	20	20	20	20	20	
N	12	33	20	20	36	64	20	20	
O	20	29	14	20	20	20	20	20	
P	31	23	3	20	26	2	20	28	
Q	20	20	20	20	26	20	20	20	
R	20	20	29	20	56	27	20	20	
S	20	20	29	20	20	20	20	20	
V	34	1	1	20	1	1	20	1	
X	20	9	14	20	16	61	20	20	

W2 - 2021-2022									
	PB2	PB1	PA	HA	NP	NA	MP	NS	
A	20	20	20	20	20	20	20	20	
C	1	1	1	20	1	1	20	1	
I	25	14	25	20	26	13	20	28	
AA	1	1	1	20	26	1	20	1	
AB	31	1	3	20	38	1	20	1	
AC	4	1	1	20	1	1	20	1	
AD	4	27	1	20	31	1	20	1	
AE	4	1	1	20	54	1	20	1	
AF	12	6	1	20	50	1	20	29	
AG	12	1	1	20	50	1	20	1	
AH	12	1	1	20	1	1	20	1	
AI	7	1	8	20	37	1	20	29	
AJ	10	1	1	20	1	1	20	1	
AK	10	1	3	20	38	1	20	1	
AL	1	1	1	20	11	1	20	1	
AM	1	1	1	20	12	1	20	1	
AN	31	1	1	20	1	1	20	1	
AO	13	1	1	20	38	1	20	1	
AP	45	31	32	20	32	1	20	28	
AQ	31	1	8	20	37	1	20	27	
AR	1	18	14	20	16	62	16	27	
AS	1	1	3	20	38	1	20	1	
AT	12	1	1	20	37	1	20	1	
AU	19	1	14	20	21	22	20	1	
AV	1	1	3	20	1	1	20	1	
AW	4	23	1	20	11	1	20	27	
AX	31	1	1	20	38	1	20	1	
AY	31	1	24	20	38	1	20	1	
AZ	1	31	1	20	12	1	20	1	
BA	13	1	1	20	1	1	20	1	
BB	31	1	43	20	43	1	20	43	
BC	45	1	43	20	37	1	20	27	
BD	31	31	8	20	26	1	20	1	
BE	4	1	14	20	26	1	20	1	
BF	13	1	1	20	11	1	20	1	

W3 - 2022-2023									
	PB2	PB1	PA	HA	NP	NA	MP	NS	
C	1	1	1	20	1	1	20	1	
I	25	14	25	20	26	13	20	28	
AB	31	1	3	20	38	1	20	1	
AF	12	6	1	20	50	1	20	29	
BB	31	1	43	20	43	1	20	43	
CA	31	1	3	20	16	1	16	1	
CB	32	1	3	20	38	1	20	1	
CC	12	1	8	20	38	1	20	1	
CD	31	1	3	20	37	1	20	29	
CE	44	1	3	20	38	1	20	1	
CF	31	1	3	20	X	1	20	1	
CG	4	1	3	20	26	1	20	1	
CH	31	1	3	20	26	1	20	1	
CI	10	1	12	20	38	1	20	1	
CJ	46	6	32	20	26	22	47	1	
CK	4	1	3	20	38	1	20	1	
CL	31	1	48	20	38	1	20	1	
CM	31	49	3	20	38	1	20	1	
CN	31	31	3	20	26	1	20	1	
CP	31	31	3	20	38	1	20	1	
CQ	31	1	3	20	37	1	20	1	
CR	31	1	3	20	38	1	20	28	
CS	34	1	3	20	38	1	20	51	
CU	xxxx	29	xxxx	20	37	1	20	27	

W4 - 2023-2024									
	PB2	PB1	PA	HA	NP	NA	MP	NS	
AB	31	1	3	20	38	1	20	1	
AF	12	6	1	20	50	1	20	29	
BB	31	1	43	20	43	1	20	43	
CH	31	1	3	20	26	1	20	1	
CK	4	1	3	20	38	1	20	1	
I	25	14	25	20	26	13	20	28	
DA	4	52	3	20	38	1	20	27	
DB	31	53	3	20	38	1	20	1	
DC	41	53	32	20	36	1	20	1	
DD	41	1	3	20	26	1	20	56	
DE	41	53	3	20	26	1	20	56	
DF	4	52	3	20	50	1	20	27	
DG	57	1	14	20	38	1	20	1	
DH	4	52	3	20	11	1	20	27	
DI	14	14	3	20	38	1	20	27	
DJ	31	52	3	20	26	1	20	58	
DK	4	1	12	20	26	1	20	1	
DL	31	1	1	20	38	1	20	1	
DM	31	52	3	20	11	1	20	27	
DN	4	60	3	20	26	1	20	27	
DO	57	1	14	20	26	1	20	1	
DP	31	1	65	20	26	1	20	1	
DQ	31	1	14	20	38	1	20	1	
DR	57	53	3	20	16	1	20	27	
DS	57	53	3	20	16	66	20	27	
DT	31	43	43	20	43	1	20	43	

W5 - 2024-2025									
	PB2	PB1	PA	HA	NP	NA	MP	NS	
BB	31	1	43	20	43	1	20	43	
DI	14	14	3	20	38	1	20	27	
I	25	14	25	20	26	13	20	28	
DA	4	52	3	20	38	1	20	27	
AF	12	6	1	20	50	1	20	29	
DT	31	43	43	20	43	1	20	43	
EA	4	14	3	20	38	1	20	27	
EB	31	14	3	20	11	1	20	29	
EC	14	52	3	20	38	1	20	27	
ED	14	31	3	20	38	1	20	27	
EE	14	14	3	20	26	1	20	27	
EF	14	14	3	20	54	1	20	27	
EG	4	14	3	20	11	1	20	27	
EI	31	14	3	20	38	1	20	27	
EJ	14	52	3	20	59	1	20	27	
EK	14	55	3	20	38	1	20	27	
EL	41	53	43	20	26	1	20	43	



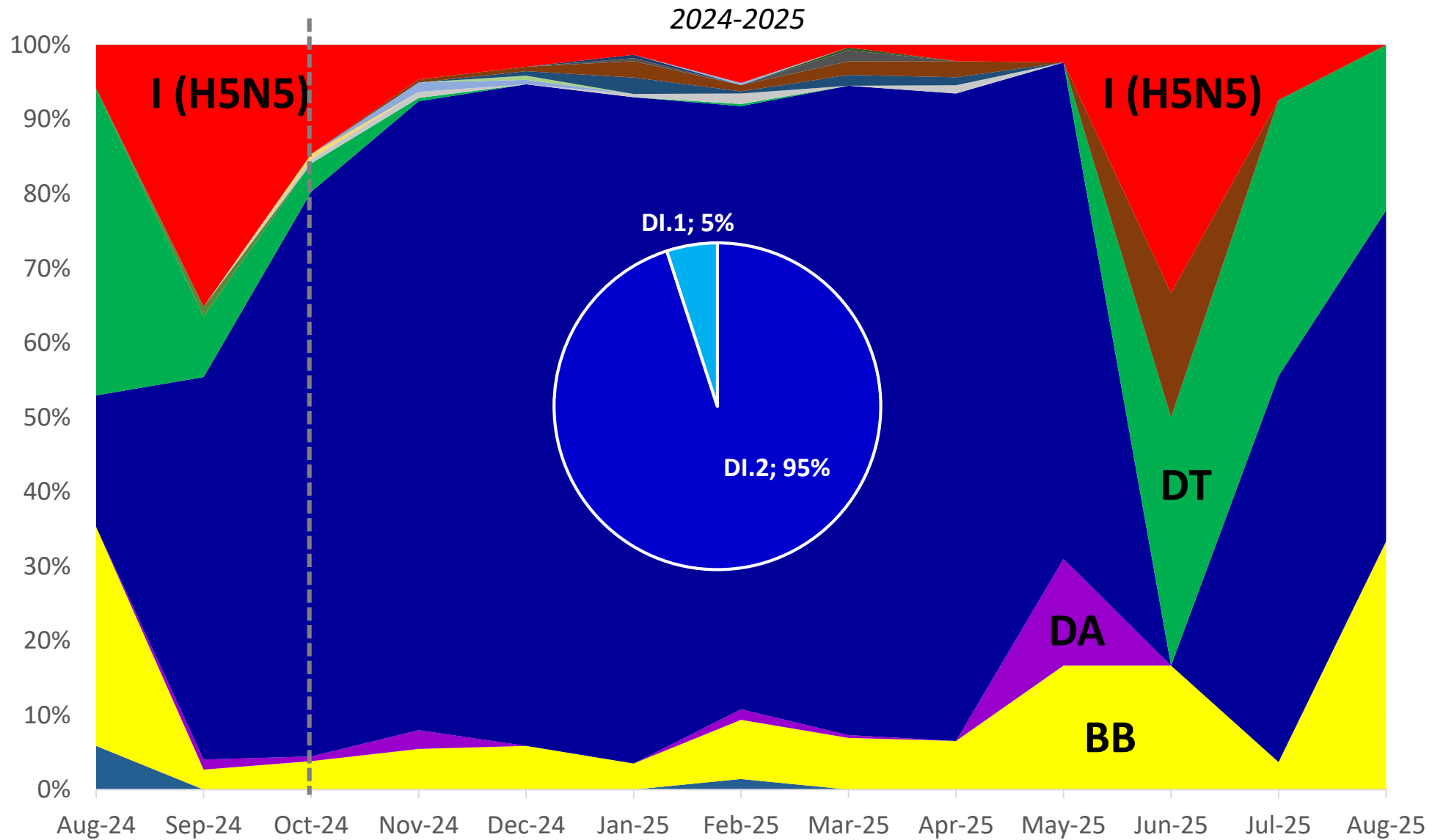
The characterized viruses during the 2024-2025 epidemiological year belong to **three major genotypes**:

- 2 H5N1 genotypes EA-2024-DI, EA-2022-BB
- 1 H5N5 genotype (EA-2021-I)

Occasional detections of novel genotypes generated from reassortments of the genotype EA-2024-DI

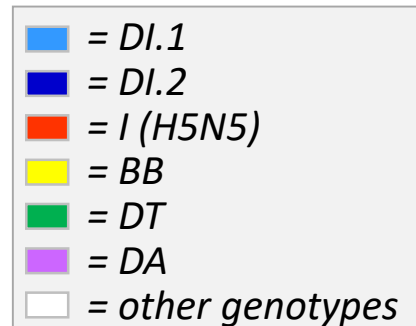
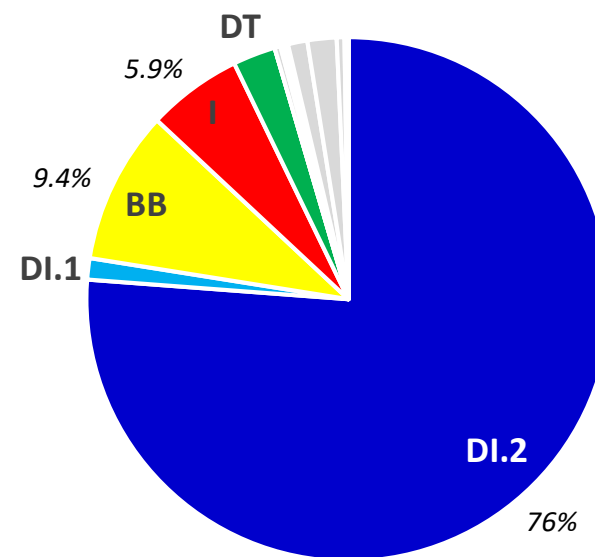
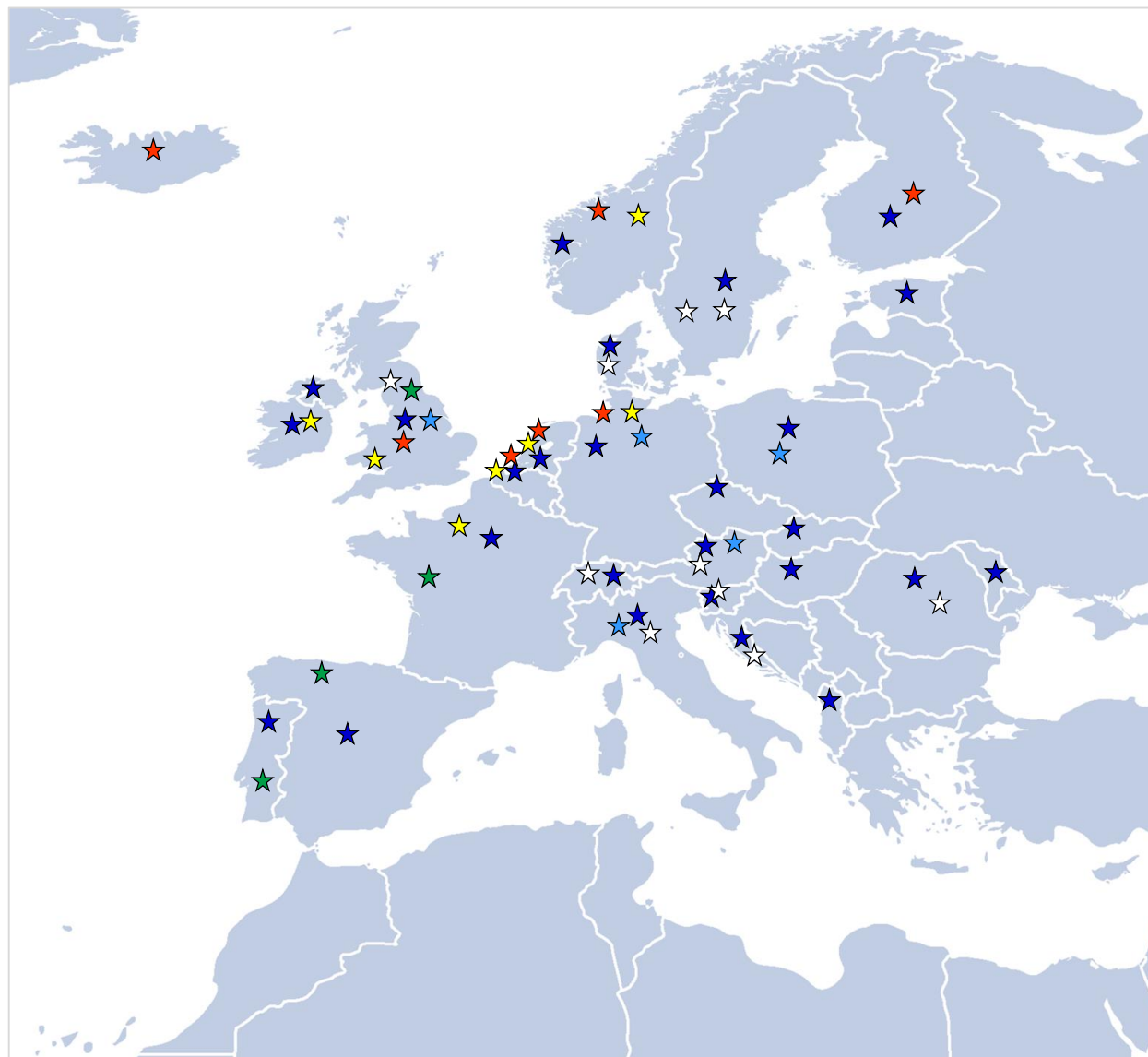


Temporal dynamics of the virus genotypes in Europe: 2024-2025



Genotype distribution in wild birds

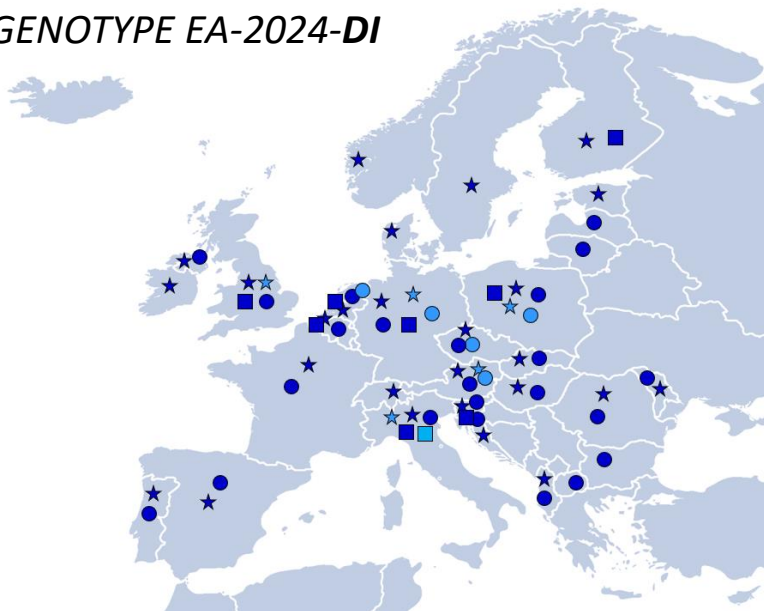
October 2024 – August 2025



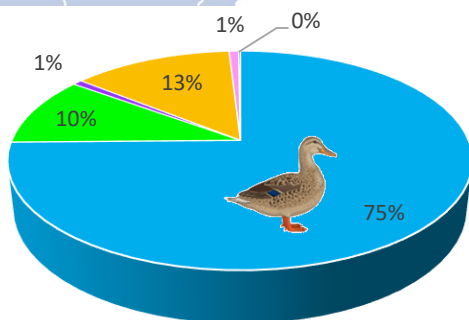
Geographic and host distribution of the genotypes, 2024-2025

☆ = wild birds ○ = poultry □ = mammal

GENOTYPE EA-2024-DI

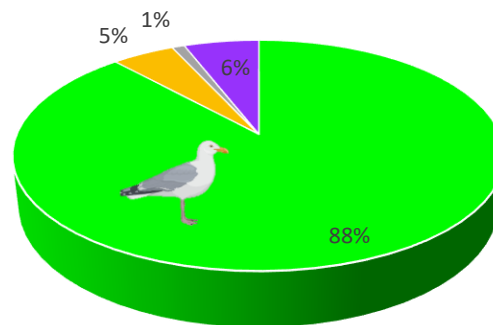
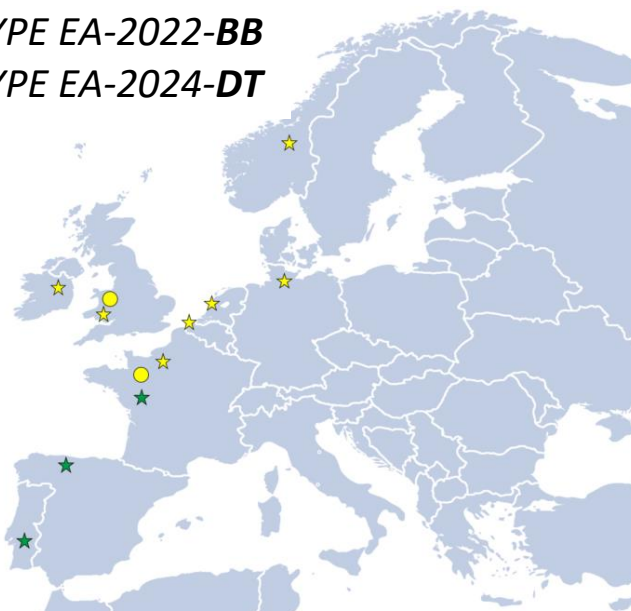


WILD BIRDS



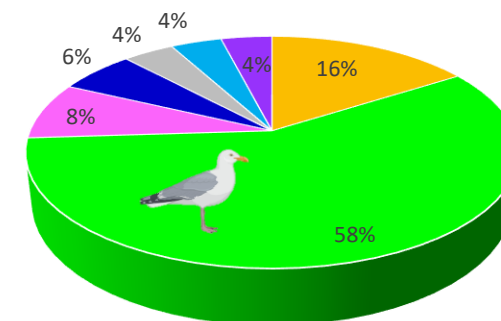
■ Anseriformes ■ Charadriiformes
■ Pelecaniformes ■ Raptors
■ Ciconiiformes ■ Podicipediformes

GENOTYPE EA-2022-BB
GENOTYPE EA-2024-DT



■ Charadriiformes ■ Raptors
■ Suliformes ■ Galliformes

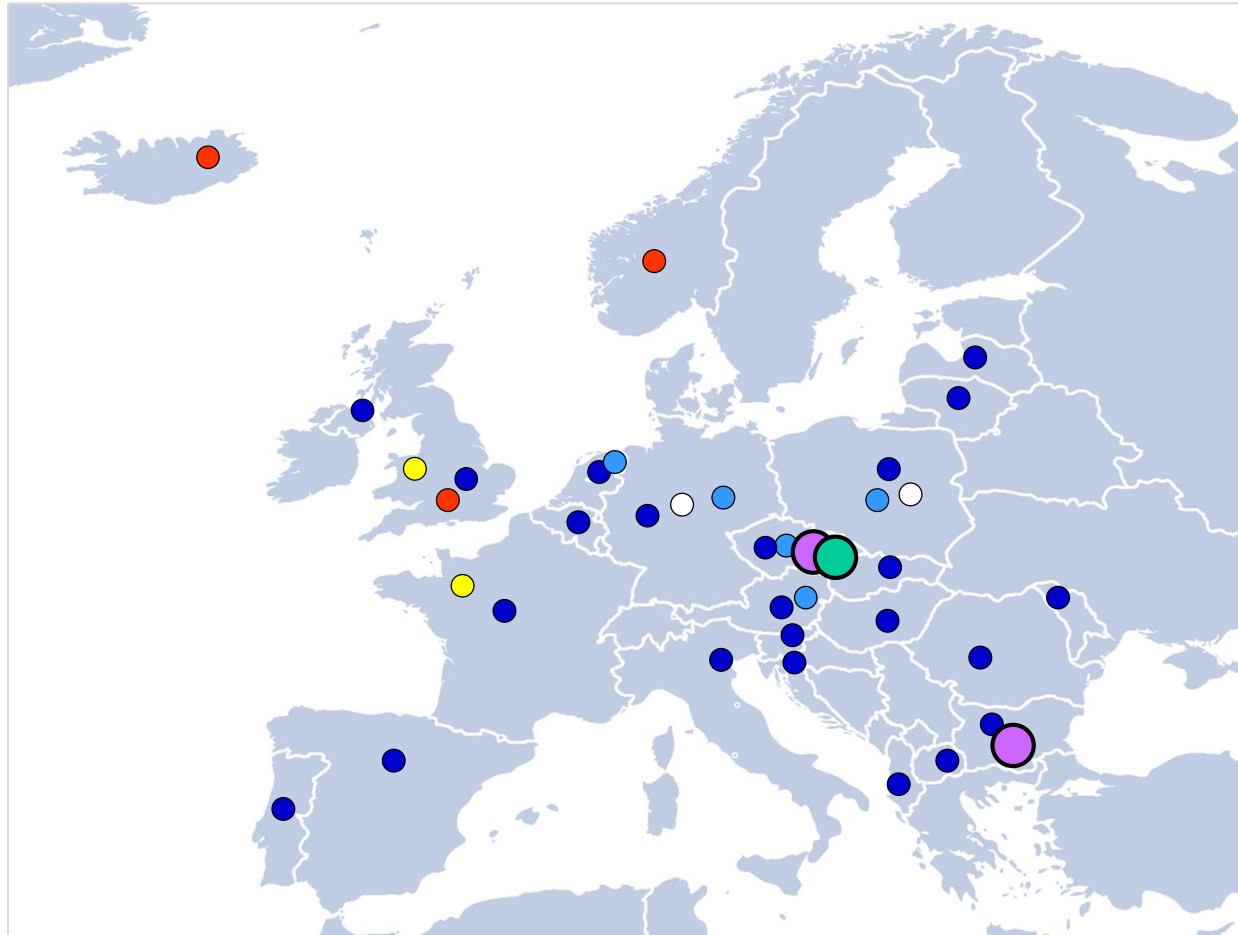
GENOTYPE EA-2021-I



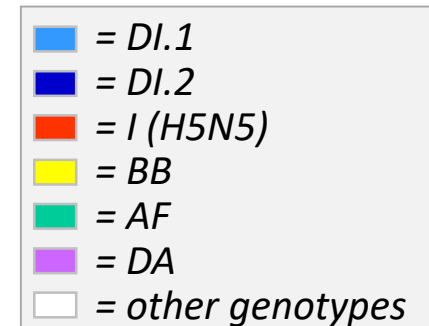
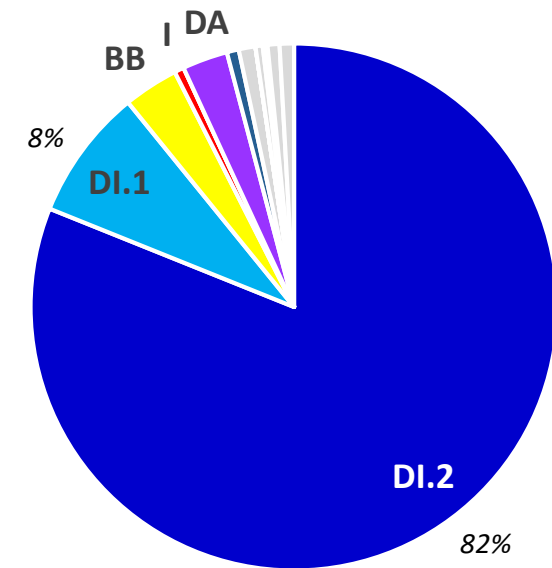
■ Raptors ■ Charadriiformes
■ Passeriformes ■ Procellariidae
■ Suliformes ■ Anseriformes
■ Galliformes

Genotype distribution in domestic birds

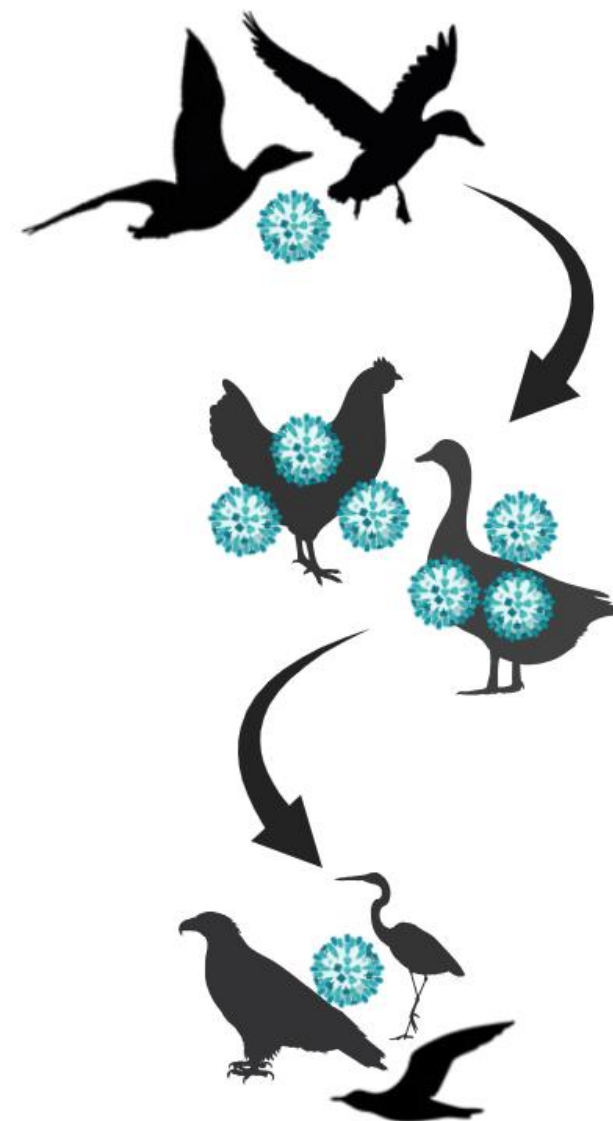
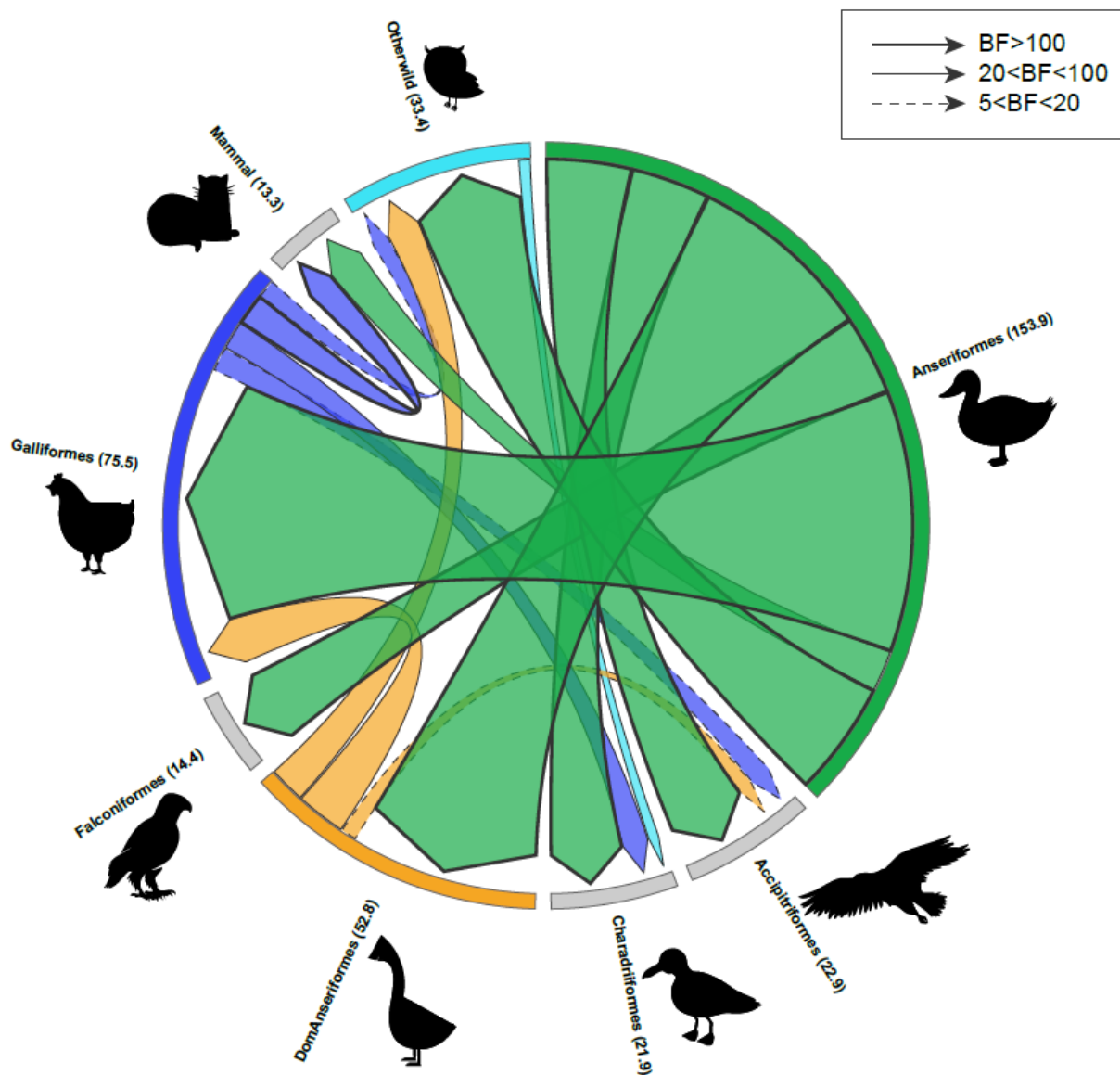
October 2024 – August 2025

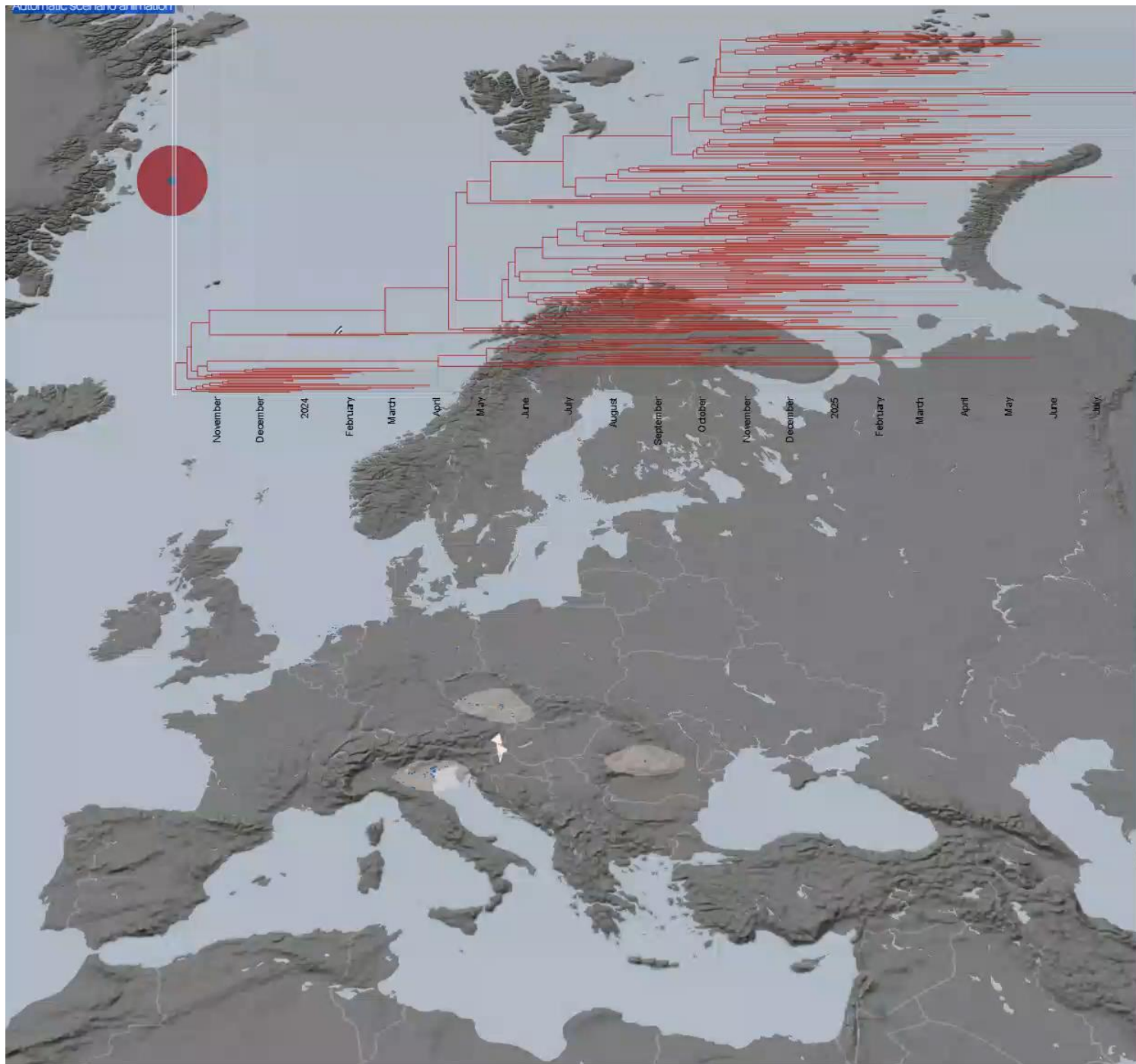
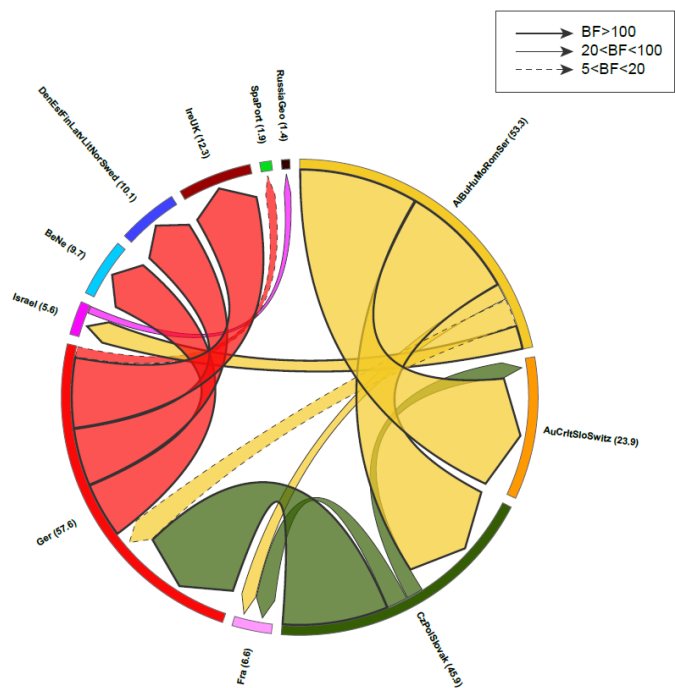
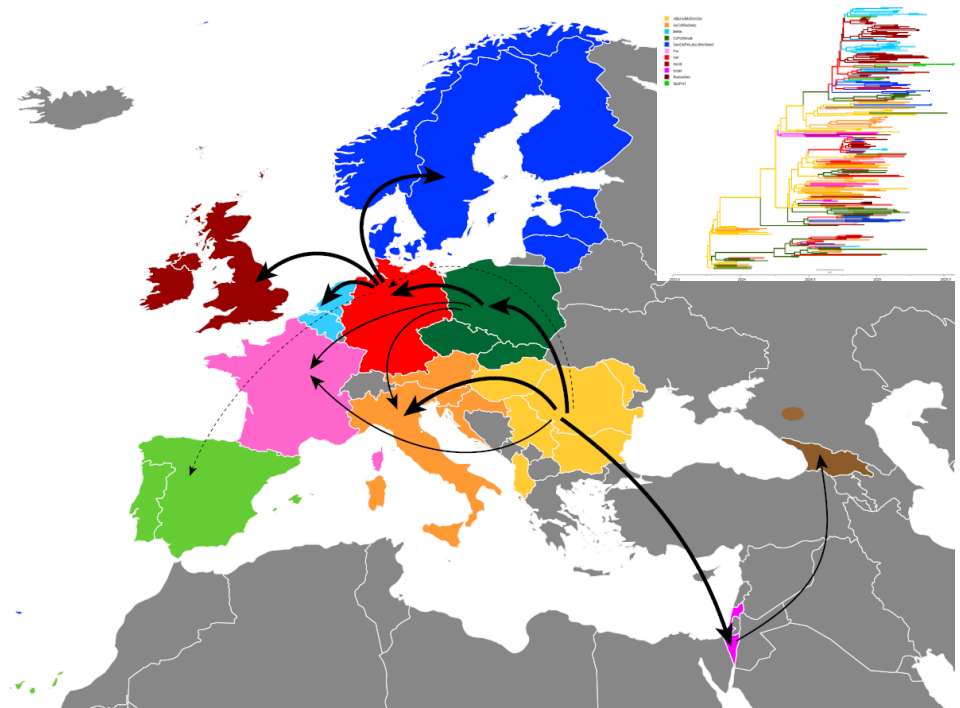


Identification in poultry in Eastern countries of two old genotypes (EA-2023-DA and EA-2020-AF), which were not detected in wild birds during the current epidemiological year in or outside other regions of Europe, suggesting undetected persistent circulation of these genotypes in an unknown ecological or domestic niche.



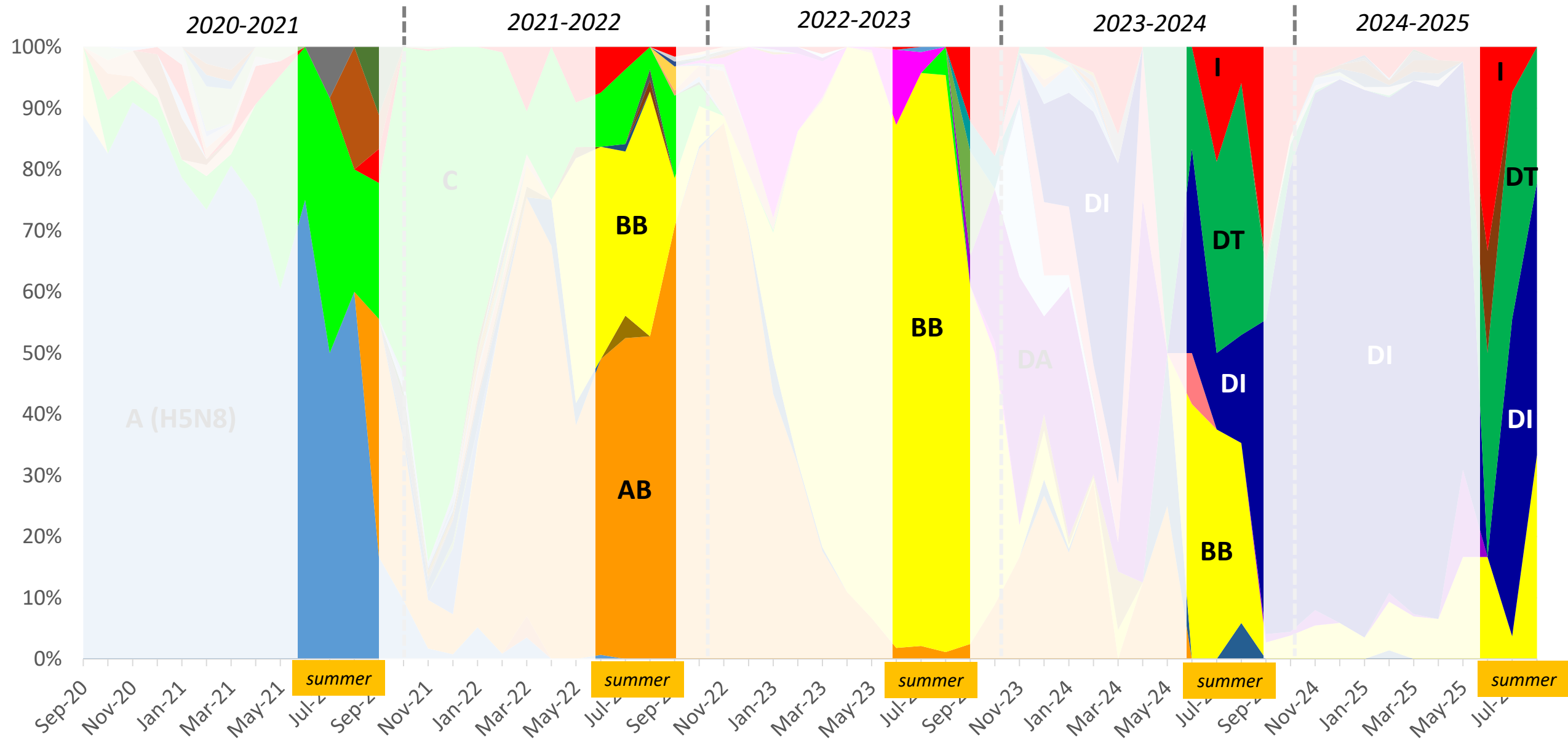
Host jumps and geographic spread of the genotype EA-2024-DI



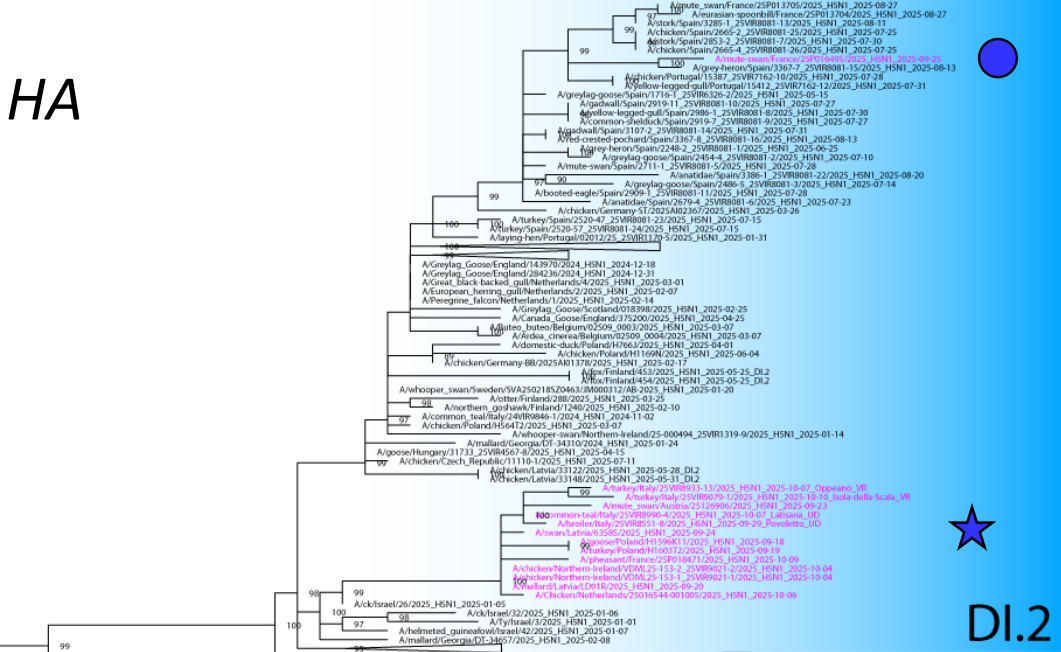




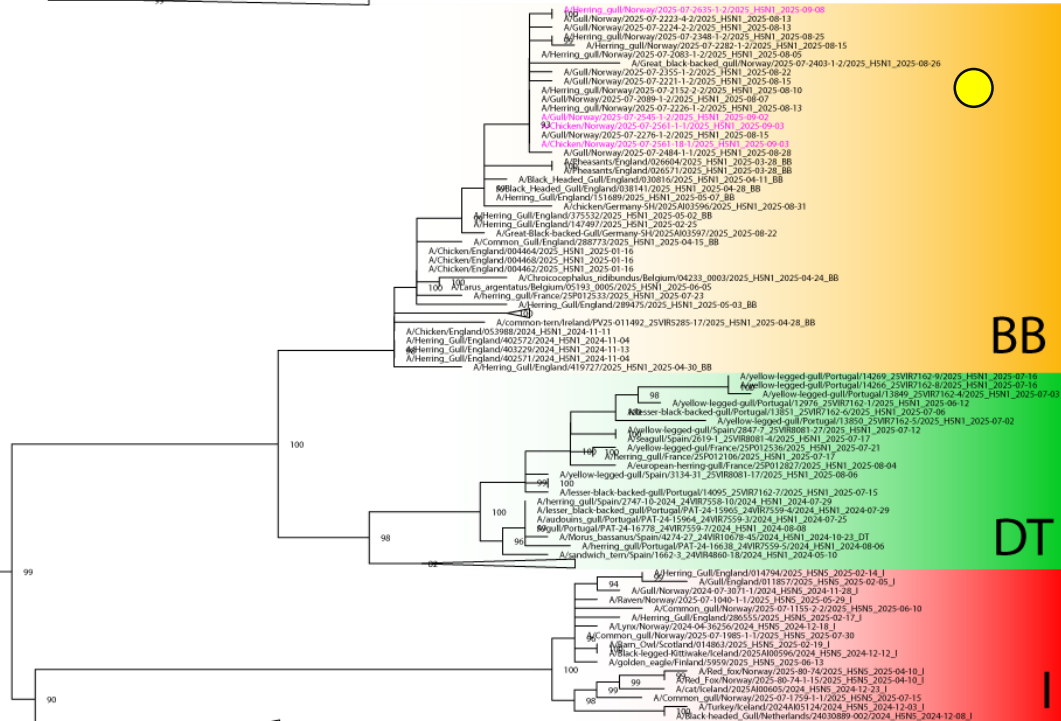
Temporal dynamics of the virus genotypes in Europe: 2020-2025



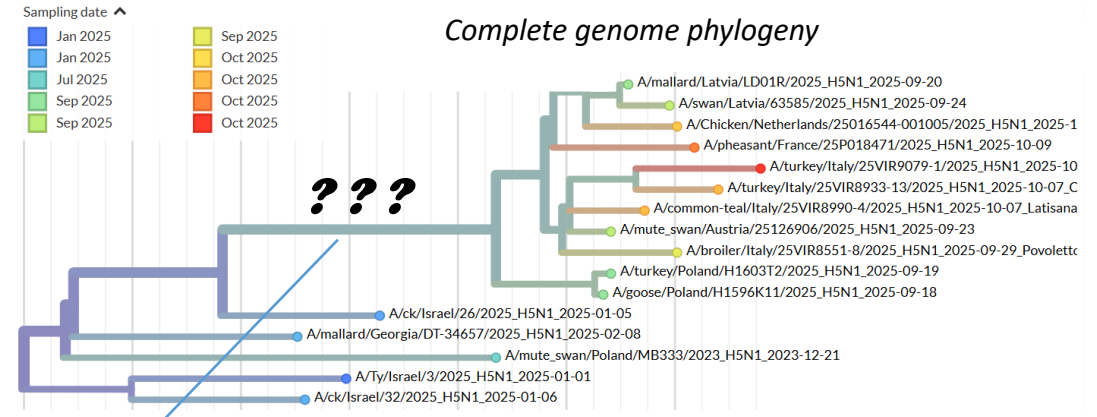
HA



DI.2



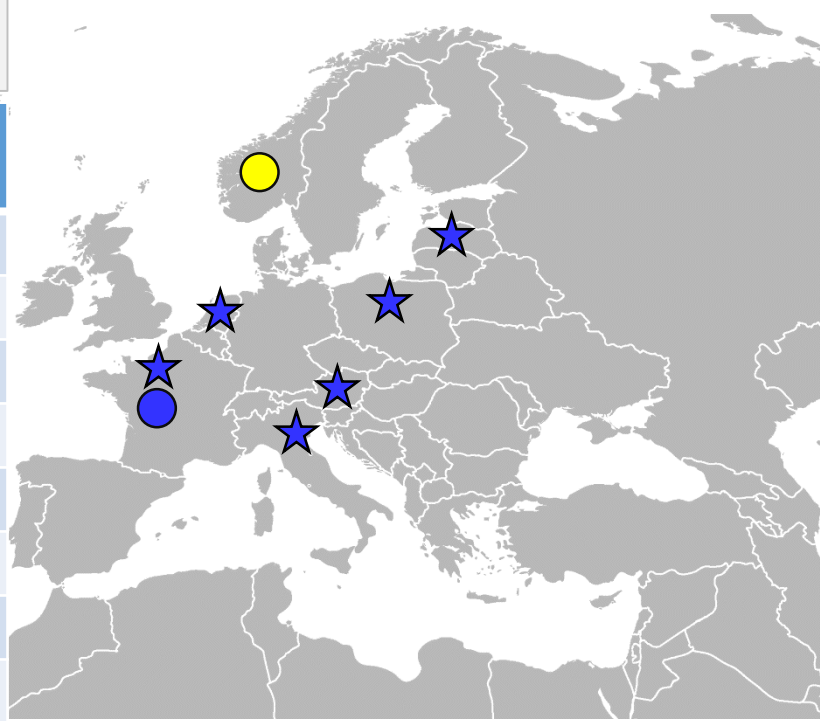
A new virus incursion?



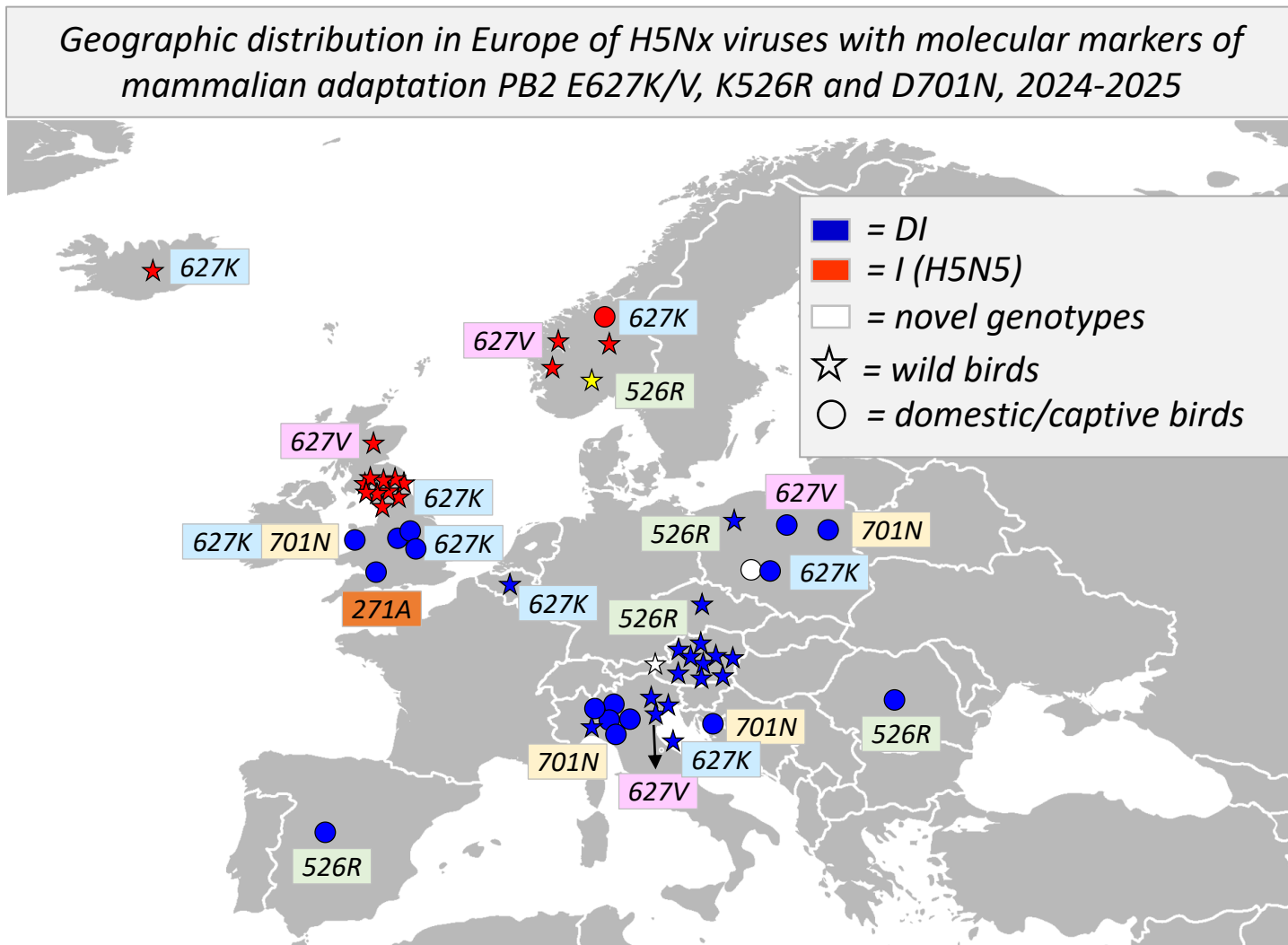
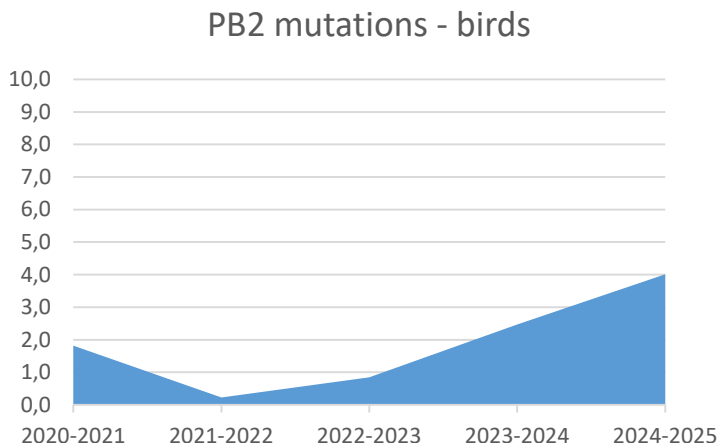
33 nt and 9 aa differences accumulated along the genome

	NT diff	AA diff
PB2	4	1
PB1	6	3
PA	7	1
HA	7	1
NP	3	0
NA	2	1
M	1	0
NS	3	2

September-October 2025

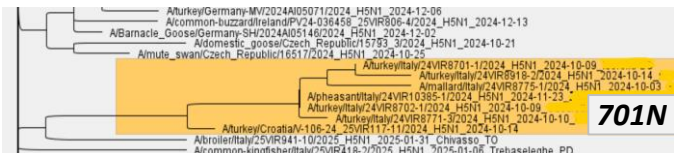


Mutations of interest in birds, October 2024-August 2025

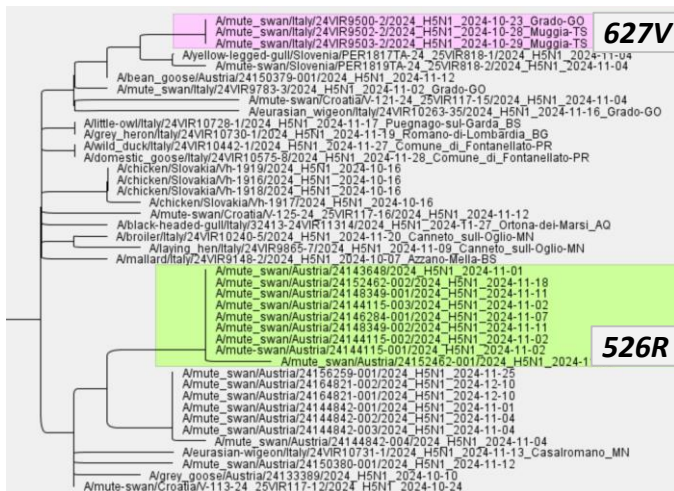


Mutations of interest in birds, October 2024-August 2025

DI



DI

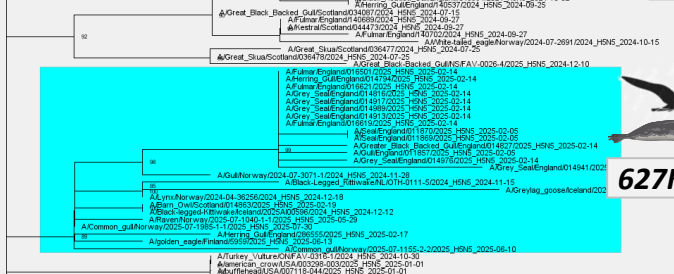


I



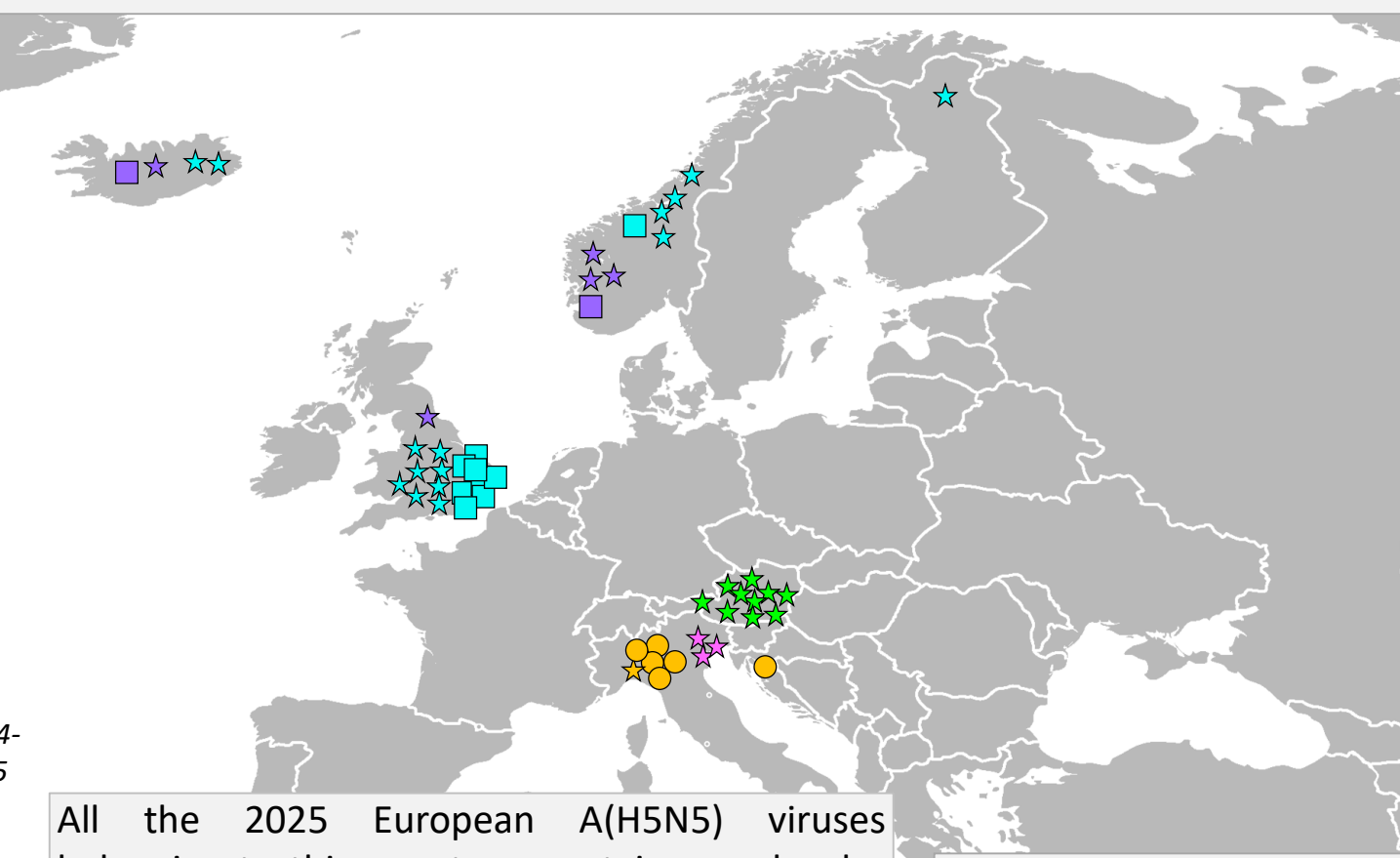
Nov 2024-
July 2025

I



Nov 2024-
July 2025

Geographic distribution in Europe of H5Nx viruses with molecular markers of mammalian adaptation PB2 E627K/V, K526R and D701N, 2024-2025



All the 2025 European A(H5N5) viruses belonging to this genotype contain a molecular marker of virus adaptation to mammals

- = mammal
- ☆ = wild birds
- = domestic/captive birds





Take home messages

- Evidence of a predominance of the EA-2024-DI genotype in 2024-2025 in Europe and the circulation of the genotypes EA-2022-BB, EA-2023-DT and EA-2021-I in separate ecological niches.
- Rapid westward diffusion of genotype EA-2024-DI at the beginning of the 2024-2025 epidemic wave, likely through *Anseriformes*.
- Poultry acted mainly as a sink host, but occasionally contributed to the virus spread to wildlife.
- Preliminary data indicates that the recent outbreaks in Europe are caused by a novel incursion of the EA-2024-DI.2 genotype.
- To date, no key mutations associated to the switch in the virus binding preference from avian to human-type receptors have been identified in the H5 collected in Europe. However, we observed an increase in the frequency of viruses collected from birds with molecular markers of mammalian adaptation in the PB2 protein. Viruses containing such mutations may have a greater zoonotic potential.



Acknowledgements

European National Reference Laboratories for AI

Aldin Lika (Albania); Sandra RevillaFernández, Irene Zimpernik (Austria); Mieke Steensels and Steven Van Borm (Belgium); Emiliya Ivanova (Bulgaria); Vladimir Savic (Croatia); Vasiliki Christodoulou (Cyprus); Alexander Nagy (Czechia); Charlotte Kristiane Hjulsager, Yuan Liang (Denmark); Tuija Gadd, Lauri Kareinen, Ari Kauppinen, Niina Tammiranta (Finland); François-Xavier Briand, Béatrice Grasland, Éric Niquex, , Audrey Schmitz (France); Timm Harder, Anne Pohlmann (Germany); Malik Peter (Hungary); Brigitte Brugger and Vilhjálmur Svansson (Iceland); Laura Garza Cuartero (Ireland); Simona Pileviciene, Egidijus Pumputis (Lithuania); Chantal Snoeck (Luxembourg); Igor Dzadzovski, Aleksandar Dodovski (Macedonia); Oxana Groza (Moldova); Monika Ballmann (Netherlands); Britt Gjerset (Norway); Anna Pikula, Katarzyna Domańska-Blicharz, Krzysztof Śmietanka and Edyta Swieton (Poland); Margarida Duarte, Teresa Fagulha, Margarida Henriques (Portugal); Iuliana Onita (Romania); Dejan Vidanovic (Serbia); Zuzana Dirbakova and Martin Tinak (Slovakia); Brigita Slavec (Slovenia); Montserrat Agüero García, María Teresa Barrios Zambrano and Azucena Sánchez (Spain); Siamak Zohari (Sweden); Claudia Bachofen (Switzerland);

Ron Fouchier from Erasmus Medical Center (Netherlands); Ashley Banyard and Holly Coombes from the Animal and Plant Health Agency (United Kingdom); Michael McMenamy from Agri-food and Biosciences Institute (Northern Ireland)

IZSVe - EURL

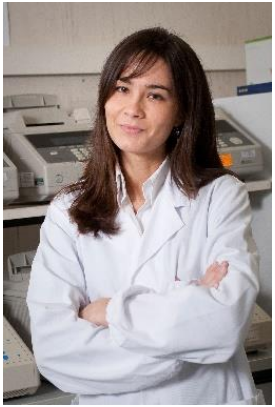
Isabella Monne, Lara Cavicchio, Marta Dianati, Edoardo Giussani, Silvia Ormelli, Angela Salomoni, Annalisa Salviato, Alessandro Sartori, Alessia Schivo, Enrico Savegnago, Maria Varotto, Gianpiero Zamperin, Bianca Zecchin, Calogero Terregino

The authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu™ Database

Phylodynamics analyses were supported by:

KAPPA-FLU HORIZON-CL6-2022-FARM2FORK-02-03 (grant agreement No 101084171)

Isabella Monne



Lillo Terregino



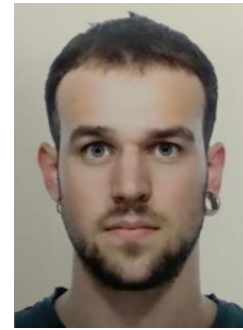
Bianca Zecchin



Marta Dianati



Enrico Savegnago



Alessia Schivo



Annalisa Salviato



*Thank you
for your attention*



Lara Cavicchio



Alessandro Sartori



Silvia Ormelli



Maria Varotto



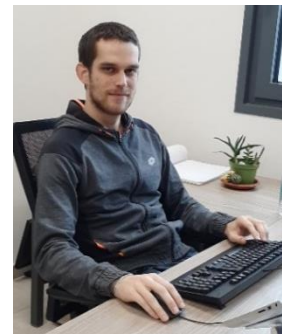
Elisa Palumbo



Angela Salomoni



Gianpiero Zamperin



Edoardo Giussani