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RESEARCH

Genetic differentiation of wild boar populations in a region affected by African swine fever

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Abstract

In the European Union, African swine fever (ASF) affects wild boar (*Sus scrofa*) populations in several Member States. Knowledge of population connectivity is important for the implementation of control measures, in particular the establishment of effective barriers. Population genetic comparisons of neighbouring populations can be very helpful in this respect. The present study investigated the genetic differentiation of wild boar in eastern Germany. This region has been affected by ASF since September 2020. A total of 1,262 wild boars from 31 hunting grounds (populations) in ASF-affected and ASF-free districts were sampled over a total area of almost 100,000 km². The study area encompassed a network of geographical factors that promote (roads, rivers, cities) or inhibit (natural areas, habitat corridors) genetic differentiation between wild boar populations. The genetic differentiation of the areas was based on 12 microsatellite markers. Three different Bayesian algorithms were used to analyse the data. The results were combined into a common approach with 9 clusters. Based on the cluster distribution in each population, the connectivity between the areas was quantified. The strongest differentiation was found along an imaginary line along the lower Elbe valley through Berlin and the A11 freeway to the Szczecin Lagoon. In contrast, the Mecklenburg Lake District and the south-east of the study area showed strong connectivity between areas. The special features of the landscapes along the lower Elbe valley, which was assessed as highly connective, and the high barrier effect of the A11 freeway in contrast to the other freeways in the study area show that barrier effects cannot be generalised in principle, but are actually determined by the circumstances of individual structures. The results of the connectivity analysis were compared with the distribution of viral lineages and variants. The genotypes of the wild boar populations and the ASFV lineages and variants showed a good explanatory approach for the observed disease dynamics in the study area. The newly gained knowledge on barriers and regionally different connectivity between wild boar populations can support considerations and measures for the containment of ASF in the affected areas by improving the understanding of wild boar dispersal dynamics.

Keywords African swine fever · Barrier · Epidemic · Genetic differentiation · Population genetics · *Sus scrofa* · Wild boar

Introduction

Wild boar (*Sus scrofa*) has proliferated and spread across Europe over the last 50 years (Massei et al. 2015; Morelle et al. 2016). Changing agro-ecosystems (Hebeisen et al. 2008), structural changes in the landscape (Morelle et al. 2016) and climate change (Markov et al. 2019) are thought to be the main causes. Their spatial and numerical distribution leads to considerable problems such as damage to crops (Schley et al. 2008) and ecosystems (Giménez-Anaya et al. 2008; Graitson et al. 2019), as well as significant threats to public health and food safety. A particular concern at present is the risk of African swine fever (ASF) spreading

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through infected wild boar populations in Germany and other European Union Member States, through humans as vectors and through the ongoing introduction of new viruses via infected wild boar from Eastern Europe (Guinat et al. 2017). Since 2007, the disease has spread from Georgia to Eastern Europe, crossing the EU border in 2014 (Sanchez-Vizcaino et al. 2015; Śmietanka et al. 2016; World Organisation for Animal Health 2017a, b). The westernmost extensions of the epidemic in Europe were Belgium (Linden et al. 2019; Pikalo et al. 2020), Germany (Sauter-Louis et al. 2020), Italy and the Czech Republic. Point introductions in Belgium and the Czech Republic have been controlled. However, the spread along the epidemic front into Germany and other countries is not entirely comparable and control measures need to be adapted (Sauter-Louis et al. 2022). It is important to address this risk situation and to take the appropriate measures to contain the disease (Keuling et al. 2016; Liordos et al. 2017; Podgórski and Śmietanka 2018; Vajas et al. 2019). Wild boar management plays a crucial role in limiting the spread of infected individuals, and thus the spread of the virus and the occurrence of enzootics in affected regions (Saegerman 2018; Petit et al. 2019; Chenais et al. 2019) (European Food Safety Authority [EFSA] 2018). This can be achieved by fencing, or increased hunting in the periphery of outbreak areas in order to prevent transmission to unaffected areas via infected individuals. At the same time, it should be enforced that infected animals in the centre of an outbreak remain in place through an initial hunting cessation, to be subsequently eliminated through population control, after measures to control the dispersal of infected animals have taken effect. Carcasses must be located and removed. In order to optimise the use of disease control measures and to focus efforts in the right places, it is necessary to understand the patterns of genetic connectivity of wild boar at regional and supra-regional level (Van der Waal et al. 2013; Hirsch et al. 2016; Podgórski and Śmietanka 2018; EFSA 2020).

Various methods have been used to map the dispersal dynamics of wild boar by GPS tagging of animals (Peris et al. 2020). Such studies provide an overview, but remain time consuming and expensive. In addition, the results are highly dependent on the individuals that have been tagged and the current situation, such as hunting pressure, weather, climate, and food supply for example (Reiner et al. 2021). Therefore, short-term studies are not able to capture the long-term effects of barriers and dispersion patterns on population dynamics.

One possible tool for representing the long-term connectivity of species in a region is the analysis of genetic differentiation. Such data are often used to demonstrate the differentiating effect of landscape resistance gradients and barriers on populations (Frantz et al. 2012; Goedbloed et al. 2013; Rutten et al. 2019). The identification of existing natural or artificial barriers could help to better target the use of resources (e.g. fencing) to contain ASF in wild boar. In a pilot study, Reiner et al. (2021) investigated the genetic differentiation between wild boar populations in Rhineland-Palatinate, Germany, as a means of recording the long-term connectivity of this species in a region threatened by ASF. Significant quantitative differences in connectivity between populations were found. By selecting populations adjacent to potential barriers, differentiating effects could be specifically attributed to individual landscape elements and the entire federal state of Rhineland-Palatinate could be divided into regions with significantly different connectivity. In this study the Rhine river and a region around the A6 freeway in the south of the federal state were identified as the main barriers (Reiner et al. 2021). Thus, knowledge of population distributions, potential barriers and movement corridors can enable the adoption of appropriate mitigation measures. By exploiting natural or artificial barriers, an affected region can be fragmented to limit the movements of wild boars and ultimately the spread of ASF.

The selected region in eastern Germany was affected by ASF in some populations along the Oder and Neisse rivers at the time of sampling between 2020 and 2022 (Forth et al. 2023). In December 2021, ASF had also just occurred in wild boar in the LUP district further west. The large number of wild boars in the region and the epidemiological characteristics of the disease raise concerns about the emergence of a long-term enzootic disease with a tendency to spread to neighbouring areas over an extended period of time.

The aim of the current study was to investigate the genetic differentiation between wild boar populations in the eastern German states of Mecklenburg-Western Pomerania, Saxony-Anhalt, Brandenburg, and Saxony. Genetic differentiation was then used to infer possible landscape barriers and preferred dispersal routes of wild boar and hence ASF.

Study area

The study area covered the eastern parts of Germany including the federal states of Mecklenburg-Western Pomerania, Brandenburg, Saxony-Anhalt and Berlin in the north, representing a lowland with Mecklenburg, Elbe Plain, Lüneburg Heath, Altmark, Brandenburg Plateau and Uckermark. The federal states of Thuringia and Saxony in the south have a low mountain range topography with the Harz Mountains, the Thuringian Forest, the Ore Mountains, Saxon Switzerland and the Zittau Mountains. The region is bordered by the Baltic Sea to the north, Poland to the east and the Czech Republic to the south. The borders of the study area to the west, east, north and south were at 10°E, 15°E, 54°N and

50°50' N, respectively. The region covered an area of about 100,000 km^2 with a north-south extent of about 450 km and an east-west extent of about 250 km.

The region has a temperate climate and lies in a transition zone between the maritime climate of Western Europe and the continental climate of Eastern Europe. Summers in the northern lowlands are cool and rainy, winters mild and stormy. In the south and east, summers are warmer and drier and winters are colder. The average annual mean daily temperature in the Mecklenburg Lake District is 8° C, with rainfall of 570 mm. The coldest month (January) is minus 1.2° C mean daily temperature, the warmest (July) 17.1° C. The climate in the south-eastern part (Upper Lusatia) is mild to warm temperate (annual average 9° C) and with high precipitation (600 mm/year).

Locally, areas with a drier climate can be found east of the Harz, Drawehn and Fläming mountains. Azonal vegetation complexes of moors, riparian forests, fens and water bodies extend along the Elbe, Havel and Spree rivers. The natural vegetation of the North German Plain consists of forests with the European beech (*Fagus sylvestris*) as the dominant species.

Altitude varies from 0 m (north coast) to 1142 m (Brocken, Harz). The predominant biome is the temperate broadleaf forest biome (deciduous forest biome), which consists of 5 different zones: the stratum tree zone, the small tree and sapling zone, the shrub zone, the herb zone, and the soil zone. The stratum zone is made up of large trees such as beech, the dominant species), oak (*Quercus spp.*), or maple (*Acer spp.*) trees. Common species in the area are wild boar, red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), red fox (*Vulpes vulpes*), and badger (*Meles meles*). The wolf (*Canis lupus*) is also currently spreading throughout the area. The human population density in 2021 varied from 69 people/km^2 (Mecklenburg-Western Pomerania) to 219/km^2 in Saxony, with the exception of Berlin with 4127 people/km^2.

Except for Berlin, the area is largely rural with about 27.8% forest, 45.4% arable land, 9.7% permanent grassland, 3% water, 11.5% settlement and traffic area. The Land Berlin has 70.6% settlement and traffic area, 4% agriculture, 17.8% forest and 6.5% water. The area is divided or bordered by 2 major rivers, the Elbe and the Oder, and more than 10 continuous and connecting freeways. Due to the topography, the freeways have few viaducts and are largely unfenced. In addition, the area includes a variety of national parks, biosphere reserves, and nature parks. The entire region supports large numbers of wild boar, especially Mecklenburg-Western Pomerania. In the 2021/2022 season, Berlin, Brandenburg, Mecklenburg-Western Pomerania, Saxony, Saxony-Anhalt, and Thuringia hunted 2.5, 90.3, 106.8, 37.1, 39.7, and 38.6 thousand wild boars, respectively. This corresponds to between 2 (Saxony and Saxony-Anhalt) and 4.6 (Mecklenburg-Western Pomerania) hunted wild boars per km^2.

Data collection

Samples for this study were collected between May 2020 and May 2022. All ASF positive samples were provided by the FLI (Federal Research Institute for Animal Health [Friedrich-Löffler-Institut], Riems, Germany) and were collected in 2021. The remaining samples originated from the stocks of the State Investigation Offices and the German Armed Forces, where they were sent in by the responsible gamekeepers of the regions for ASF surveillance purposes. All samples were taken from hunted animals or from dead wild boars. No live animals were sampled, nor were any animals hunted or otherwise killed specifically for this study.

Samples from 1,262 wild boars from 31 hunting grounds (Supplementary Table 1; Fig. 1) were included in this project, of which 10 hunting grounds were affected by ASF. Samples were strategically selected, to cover a network of potential barriers, such as freeways, settlements, rivers, and areas between nature reserves. The groups of animals on the hunting grounds then formed the units of the evaluation referred to as "populations". This applies both to the population-based genetic parameters (Fis, Fst, …) and to the analysis of the distribution of genetic clusters calculated using Bayesian methods. The number of analysed wild boars per population ($\bar{x} = 41$) provided a reliable genetic characterisation of the populations (Reiner et al. 2019). Lower numbers of animals in three individual comparison groups resulted from the distribution of ASF-positive and ASF-negative animals within the population.

DNA extraction and genotyping

DNA was extracted using a commercially available kit (Viral RNA Mini Kit, Qiagen, Germany). From each sample 80 µl of blood was processed according to the manufacturer's instructions. DNA was eluted in 60 µl of RNAse-free water. DNA concentration was determined photometrically using a Qubit Flex fluorometer (Thermo Fisher Scientific, Germany) and adjusted to a concentration of 5 ng/µl with RNAse-free water. This DNA concentration gives the best results in capillary electrophoresis. The presence of high molecular weight DNA was confirmed by agarose gel electrophoresis. Each DNA extraction was accompanied by a blank extraction without sample material, which we used as a negative control in polymerase chain reaction (PCR). For each PCR analysis the same wild boar sample was used as positive control. The sample was then used as a standard in capillary electrophoresis (see below). The wild boar was

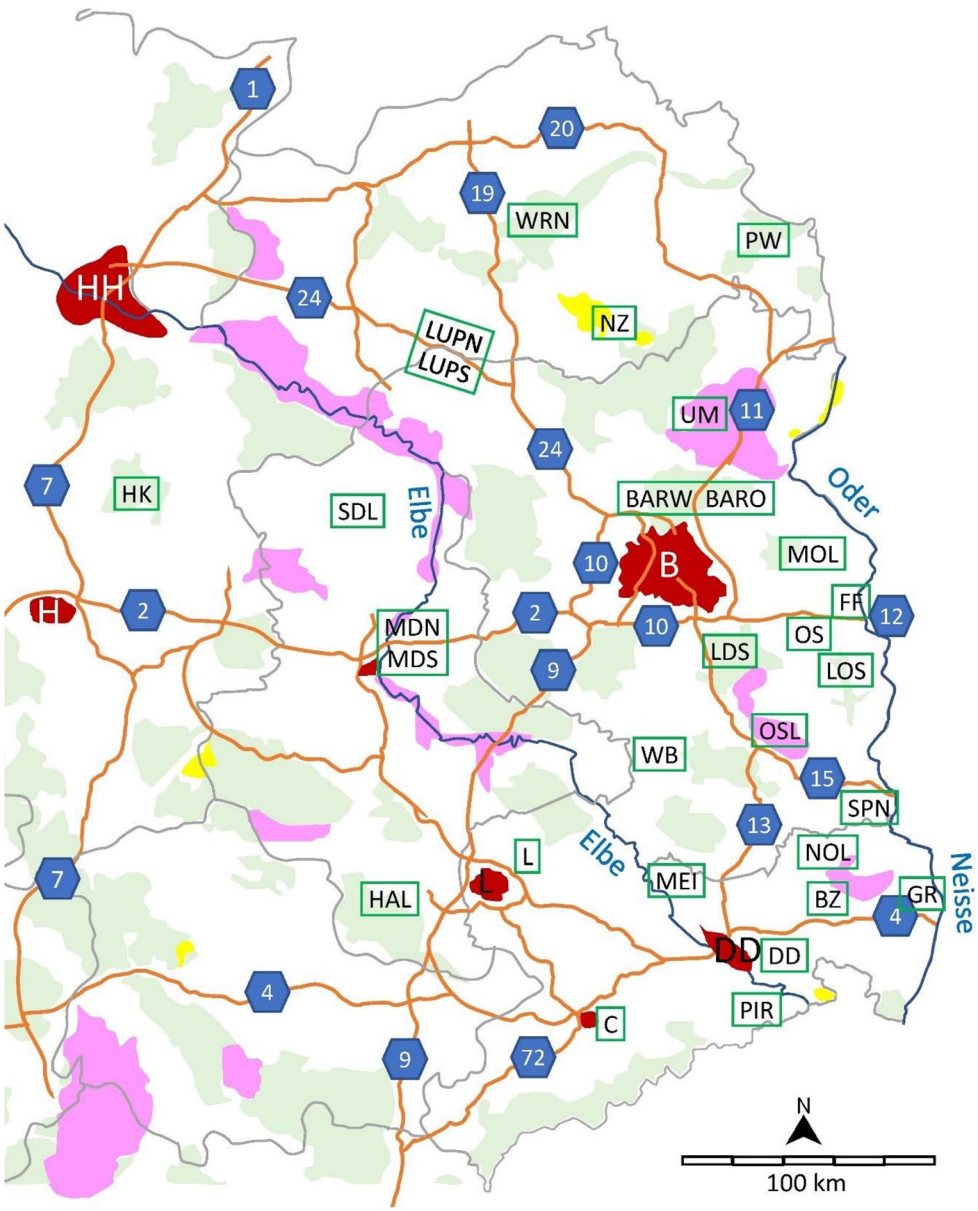

Fig. 1 Location of the populations: Bergen (HK), Ludwigslust-Parchim (LUP), Waren, Hubertushof (WRN), Neustrelitz, Mecklenburgische Seenplatte (NZ), Uckermark (UM), Jägerbrück (PW), Nettelgrund bei Jägerbrück (PW), Barnim/Berlin (BAR), Märkisch Oderland (MOL), Wittenberg (WB), Storkow, Dahme-Heideseen (LDS), Frankfurt/Oder (FF), Oder/Spree (OS), Grimma, Leipzig (L), Dahme-Spreewald (LOS), Oberspreewald/Lausitz (OSL), Oder-Spree-Neiße, Eisenhüttenstadt (EH), Meißen (MEI), Chemnitz (C), Dresden (DD), Bautzen (BZ), Upper Lusatia (NOL), Görlitz-Spree-Neiße (SPN), Sächsiche Saxon Switzerland (PIR), Görlitz (GR), Magdeburg (MD), Halle (HAL), Stendal (SDL); numbers on blue fields: freeway numbers; orange lines: freeways; blue lines: rivers; grey lines: state borders; major *cities* (dark red): B = Berlin; HH = Hamburg; H = Hannover; L = Leipzig; DD = Dresden; C = Chemnitz; *national parks* (yellow), *biosphere reserves* (pink), *nature parks* (green)

genotyped using 14 microsatellites. Primers were purchased from Biomers (Germany) and combined in 2 multiplex PCRs (Supplementary Table 2). PCR was performed in a volume of 10 µl consisting of 5 µl of 2× multiplex mastermix (Qiagen, Germany), 4 µl of primer mix, and 1 µl (5 ng) of extracted DNA. After an initial denaturation step of 15 min DNA was amplified in 26 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C (multiplex PCR 2 at 60 °C) for 90 s, and extension at 72 °C for 30 s. After a final step at 60 °C for 30 min, the PCR reactions were cooled down to 4 °C.

Capillary gel electrophoresis

One microlitre of the fluorescently labelled PCR product and 0.375 µl DNA Size Standard 500 Orange (Nimagen, The Netherlands) were added to 12 µl Hi-Di-formamide (Thermofisher Scientific, Germany) and electrophoresed on an ABI 310 capillary sequencer. All homozygous samples were analysed twice and allele sizes were determined using the Peakscanner 2.0 software (Thermofisher Scientific, Germany). Allele sizes from the positive control sample (see above) were averaged over 10 runs and used as standard. In each run the positive control sample was electrophoresed along with the other samples. Run-to-run variation in allele size was monitored by comparing the allele sizes of the

positive control sample with those of the standard. Deviations between the two were used to correct the allele sizes of the other samples.

Analysis of population genetic parameters

Population genetic analyses were performed in R (R Core team 2017). The frequencies of null alleles were calculated using the function null.all implemented in the R package PopGenReport version 3.0.4 (Gruber and Adamack 2015). As the frequency of missing data was < 5%, null allele frequencies were estimated using the method described by Brookfield (1996). To calculate the 95% confidence interval (CI), 1,000 bootstraps were used. If the 95% CI includes zero, the null allele frequencies are not significantly different from zero.

Hardy-Weinberg equilibrium (HWE) was tested in each population and in the full dataset using the hw.test function implemented in the R package pegas version 0.12 (Paradis 2010). The test was performed as an exact test based on Monte Carlo permutations ($n = 1{,}000$) of alleles (Guo and Thompson 1992). Private alleles and uniformity of allele distribution were determined using functions implemented in the R package poppr version 2.8.3 (Kamvar et al. 2014).

Population genetic parameters (number of alleles/population, percentage of alleles/locus/population, mean number of alleles, allelic richness, effective number of alleles, observed heterozygosity, expected heterozygosity, inbreeding coefficient Fis) were calculated using the divBasic function implemented in the R package diveRsity version 1.9.90 (Keenan et al. 2013). Inbreeding coefficient values were presented with their 95% CI obtained after 1,000 bootstrap iterations. Using the same R package (diversity version 1.9.90), pairwise population differentiation was calculated using Fst (Weir and Cockerham 1984) as the population statistic.

Population differentiation was assessed using one non-spatial (STRUCTURE 2.3.4, Pritchard et al. 2000) and two spatial (TESS 2.3, Francois et al. 2006; Chen et al. 2007; BAPS 6.0, Corander and Marttinen 2006) methods, all using individual-based Bayesian clustering algorithms to detect genetic discontinuities. In principle, all available wild boars were used to detect genetic clusters. The distributions of the clusters in the related animal groups per population (where all individuals from one hunting ground were designated as a population) were then used to visualize and quantify the similarities between these populations.

Hierarchical STRUCTURE analysis was performed to detect underlying genetic structures at a finer resolution. This was done by using the clusters inferred from the first round as input to a further STRUCTURE analysis were used. These steps were repeated until no further clustering could be detected. Population admixture and correlated allele frequencies were then assumed. Simulations were then run with 200,000 MCMC iterations after a burn-in of 100,000 were performed. Finally, K was varied from 1 to 10 with ten independent runs/K and the optimal K was determined using Structure Harvester 0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester/, *accessed on 23.12.2020*). The R package pophelper (Francis 2017; https://github.com/royfrancis/pophelper, accessed on 23.12.2020) was used to determine the population assignment probability of each individual across all simulations and to visualize population connectivity. Additionally, TESS was run with relaxed parameters (1,200 sweeps with a burn-in of 200 sweeps, maximum number of clusters Kmax fixed at 10, 10 runs for each K) in a first run to determine the optimal number of clusters from the lowest deviance information content value. Then 100 independent runs were performed at the optimal K with 50,000 sweeps after a burn-in of 10,000 sweeps were performed. All runs were performed under the assumption of admixture. Finally, BAPS was run with the option of spatial clustering of individuals.

Individual assignment probabilities as a result of the Bayesian clustering approaches to visualize cluster membership of individuals from populations are presented as pie charts. To do this, the cluster assignment probabilities of individuals from each population were averaged and expressed as a percentage.

Furthermore, individual assignment probabilities from STRUCTURE were used in a generalized linear model (IBM-SPSS version 27, IBM, Munich, Germany) to quantify the genetic differentiation between neighbouring populations and to investigate the relevance of the differentiating regions. This model was used to test whether the distribution of the assignment probabilities to 2 clusters ($K = 2$), determined with STRUCTURE, differed significantly between the populations. Pairwise comparisons and Bonferroni-based corrections of results were performed. P-values ≤ 0.05 were considered as statistically significant.

In order to highlight the commonalities between the results of the three Bayesian methods and to adjust for differences, the STRUCTURE, BAPS and TESS clusters were used in a joint cluster analysis (Hierarchical Cluster, IBM-SPSS V.27, Munich, Germany). This resulted in 9 significant clusters. The distribution of the clusters in the populations is presented as pie charts. In addition, the differentiation between the populations was quantified by their pairwise deviation in the summed up individual cluster assignment probabilities of clusters 1–9. This resulted in a minimum of 0 for absolute equality of cluster assignment probabilities in the 9 clusters and 200 for absolute inequality between the two populations. By dividing by 2, the deviation was output as a percentage (0-100).

Isolation by distance can significantly affect the results of Bayesian clustering methods (Perez et al. 2018). Therefore, isolation by distance was assessed using a Mantel test. Slatkin's linearised Fst values (Fst/[1-Fst]) were used as population genetic metrics. The Mantel test and the Mantel correlogram analysis were performed using the R package vegan (version 2.5.7) (Oksanen et al. 2020) to detect spatial patterns in the overall Mantel correlation within geographic distance classes (Diniz-Filho et al. 2013).

Quantifying effects of selected barriers

The pairwise deviation in the summed up individual cluster assignment probabilities between populations, which were calculated on the basis of the joint cluster analysis was further analyzed in a univariate linear analysis of variance (IBM-SPSS V27, Munich, Germany) with the geographical distance between populations as a covariate in order to quantify the effects of individual barriers (e.g. freeways). For this purpose, the pairwise deviations of populations on the same side of the barrier were compared with those of pairs separated by the barrier. The areas of the freeways A9, A11, A12, A15 and the area of the freeway A24 together with the Elbe Plain were used as barriers, because for these areas sufficient pairwise comparisons were available for a meaningful analysis. The populations included for each barrier are listed below: A9 (WB, L, MEI, C, HAL, MDN and MDS), A11 (MOL, BARO, FF, WRN, NZ, PW, UM and BARW), A12 (BARO, MOL, FF, OS, LDS and LOS), A15 (LDS, OS, LOS, WB, MEI, NOL, BZ and SPN) and A24 in combination with the Elbe Plain (HK, SDL, MDN, LUPN, WRN, NZ, UM, BARW). LUPS was not included in the analysis of the A24/Elbe area, as the population was located between the A24 and the Elbe. For each barrier there were therefore 13 comparisons on the same side and 15 comparisons across the barrier were therefore available. Only for the A12 were there fewer comparisons available (9 instead of 13 and 12 instead of 15).

ASFV detection and variant analyses

Nucleic acids were extracted using the manual QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations or the NucleoMag Vet kit (Macherey Nagel) on a KingFisher automated 96-well extraction platform (Thermo Fisher). A commercial real-time PCR was then used to detect ASFV genomes (virotype ASFV 2.0, Indical Bioscience, Leipzig, Germany). All PCRs were performed on a Bio-Rad C1000TM thermal cycler (BIO-RAD, Hercules, California) using the CFX96TM real-time system from the same manufacturer. The qPCR results were initially recorded as quantification cycle (cq) values.

Marker identification and genetic typing were performed as previously described (Forth et al. 2023). Briefly, the following genomic regions allowed lineage identification: A240L mutation (lineage II); MGF505-4R mutation (lineage III); MGF 360-15R mutation (lineage IV) and E199L mutation (lineage V). Conventional PCR was performed using the above region-specific primer pairs and AgPath-ID™ One-Step RT-PCR reagents (Thermo Fisher Scientific) according to the manufacturer's instructions in a 12 µl reaction on a C1000 thermal cycler (Biorad, Hercules, USA). PCR reactions were then sent to Microsynth Seqlab GmbH (Göttingen, Germany) for analysis. The service included PCR clean-up and Sanger sequencing.

Results

We started with 14 microsatellite markers. One of these, Sw951 was monomorphic in 16 of the 31 populations studied and was therefore excluded from further analysis. Null allele frequencies of markers significantly different from zero were detected in 15 out of the 31 populations. The most prominent marker prone to null alleles was Sw0155 with null allele frequencies ranging from 4.4 to 30.3%. Therefore, this marker was also removed from the data set. All other null allele frequencies significantly different from zero were distributed across different markers and different wild boar populations and ranged from 6.2 to 26.4%.

Markers Sw632 and Swr1941 had the highest ($n=23$) and marker Sw72 the lowest number of alleles ($n=8$). This is also reflected in the informativeness (In) of the markers (Rosenberg et al. 2003) which was highest for SW936 (In $=0.272$) and lowest for Sw72 (In $=0.125$).

A total of 59 private alleles were found, most of which were detected for the markers Swr1941 ($n=11$), S0225 ($n=7$), Sw632 ($n=7$), Sw911 ($n=6$) and S0227 ($n=5$). Private alleles were predominantly distributed among the wild boar populations LOS ($n=20$), FF ($n=13$), and MOL ($n=7$). Each population contained at least one private allele.

Alleles of marker Sw72 ($n=8$) were most evenly distributed across all populations (evenness $=0.88$) over all populations whereas allele frequencies of marker S0225 with 19 alleles varied considerably (evenness $=0.44$). Differences between Ho and He were statistically significant for the whole population ($P=0.00049$) (Table 1). In 7 out of 31 populations the observed heterozygosity (Ho) of markers was higher than the expected heterozygosity (He). All markers showed a consistent deviation from HWE in the whole population. Markers S0225, S0227, and S0226 were out of

Table 1 Population genetic parameters for the wild boar populations in East Germany, 2020–2022

Population	Shortcut	ASF	*N*	A	Na	%	Ar	Ho	He	Fis	Fis_Low	Fis_High
Bergen, South Lüneburg Heath, LSAX	HK	0	57	66	5.5	37.65	3.02	0.55	0.59	0.068	0.009	0.122
Hubertushof, Waren, MVP	WRN	0	51	59	4.9	33.64	2.87	0.58	0.55	-0.048	-0.099	0.006
Neustrelitz, MVP	NZ	0	18	54	4.5	31.87	3	0.61	0.59	-0.025	-0.09	0.038
Uckermark, BB	UM	0	28	60	5	35.26	3.01	0.6	0.59	-0.013	-0.083	0.053
Jaegerbrück, Hinterland, MVP	PW	0	181	78	6.5	45.92	2.99	0.56	0.57	0.024	-0.006	0.053
Märkisch Oderland, BB	MOL-	0	14	49	4.1	29.62	2.87	0.58	0.59	0.031	-0.073	0.118
Märkisch Oderland, BB	MOL+	1	28	76	6.3	41.63	3.11	0.56	0.63	0.11	0.011	0.215
Wittenberg, Fläming, BB	WB	0	61	71	5.9	40.84	3.12	0.58	0.62	0.074	0.027	0.118
Storkow, Dahme-Heideseen, BB	LDS	0	52	59	4.9	33.83	3.01	0.59	0.6	0.003	-0.044	0.052
Grimma, Leipzig, SAX	L	0	10	57	4.8	34.29	3.07	0.62	0.6	-0.029	-0.19	0.109
Oder, Dahmen, Spreewald, BB	LOS	1	120	106	8.8	61.27	3.19	0.6	0.63	0.057	0.014	0.102
Oberspreewald, Lusatia, BB	OSL	0	28	64	5.3	38.41	3.11	0.58	0.63	0.071	0	0.14
Meissen, Lusatian Plateau	MEI	0	66	74	6.2	42.97	3.17	0.59	0.62	0.056	0.018	0.097
Chemnitz, SAX	C	0	10	59	4.9	33.28	3.34	0.67	0.68	0.005	-0.141	0.122
Ludwigslust, MVP, north M24	LUPN	0	14	53	4.4	30.83	2.95	0.59	0.58	-0.008	-0.101	0.067
Ludwigslust, MVP, south M24	LUPS	0	8	40	3.3	24.24	2.52	0.5	0.48	-0.048	-0.249	0.091
Dresden, SAX	DD	0	28	65	5.4	38.15	3.24	0.63	0.63	-0.01	-0.08	0.054
Bautzen, SAX	BZ	0	60	87	7.2	50.7	3.28	0.6	0.62	0.033	-0.02	0.083
Oberlausitz, Sax	NOL	0	56	77	6.4	44.68	3.15	0.6	0.62	0.044	-0.005	0.096
Görlitz, Spree-Neisse, SAX	SPN	1	50	78	6.5	44.81	3.22	0.59	0.65	0.091	0.03	0.154
Saxony Switzerland	PIR	0	61	76	6.3	43.95	3.29	0.62	0.65	0.045	-0.003	0.092
Görlitz, Upper Lusatian Plateau, SAX	GR-	0	14	62	5.2	35.85	3.34	0.6	0.68	0.119	-0.008	0.242
Görlitz, Upper Lusatian Plateau, SAX	GR+	1	96	92	7.7	52.95	3.3	0.61	0.64	0.053	0.015	0.093
Halle, SA	HAL	0	13	59	4.9	34.15	3.18	0.62	0.66	0.063	-0.075	0.2
Stendal, Elbe Plain, SA	SDL	0	11	56	4.7	32.65	3.11	0.55	0.6	0.086	-0.037	0.187
Barnim, BB, west M11	BARW	0	18	56	4.7	33.4	2.97	0.59	0.58	-0.026	-0.149	0.093
Barnim, BB, east M11	BARO	1	4	35	2.9	21.02	2.41	0.38	0.55	0.319	-0.194	0.544
Oder/Spree, Spreewald, BB	OS	1	64	101	8.4	57.97	3.29	0.6	0.65	0.088	0.026	0.15
Frankfurt/Oder, BB	FF	1	21	65	5.4	38.6	3.2	0.61	0.62	0.021	-0.108	0.14
Magdeburg, SA, north M2	MDN	0	12	57	4.8	34.36	3.03	0.56	0.61	0.082	-0.076	0.228
Magdeburg, SA, south M3	MDS	0	8	45	3.8	27.1	2.78	0.51	0.55	0.074	-0.103	0.201
Min			4	35	2.9	21.02	2.41	0.38	0.48	-0.05	-0.235	0.006
Max			181	106	8.8	61.27	3.36	0.67	0.68	0.319	0.031	0.562
Mean			41	65.7	5.47	38.255	3.071	0.582	0.61	0.045	-0.057	0.1312
Standarddeviation			38	16.3	1.35	9.0865	0.213	0.05	0.04	0.069	0.073	0.099

[a]: number of samples; [b]: number of alleles per population; Na: mean number of alleles per population; %: percentage of alleles per locus per population; Ar: allelic richness; Ho: observed heterozygosity; He: expected heterozygosity; Fis: fixation index; Fis_Low: lower (2.5%) confidence interval for fixation index; Fis-High: upper (97.5%) confidence interval for fixation index; Mean: mean values for all populations; SD: standard deviation for the populations; Column 1: populations (see also Fig. 1); ASF: 0 = population free of ASF, 1: population affected by ASF.

HWE in 7, 5, and 5 populations, respectively. Marker SW24 was in HWE in all wild boar populations.

Based on an average of 41 animals/population (4-181), the mean number of alleles within the population was 65.7 (35 to 106). The highest mean number of alleles (Na = 8.4 and 8.8) was observed in the populations FF and LOS in the Spreewald Forest. Populations with low sample numbers due to few animals on one side of a freeway or with/without ASF had low numbers of alleles (Table 1).

Allelic richness (Ar) was very close in each of the populations. The observed heterozygosity (Ho) varied between 0.38 and 0.67. Fis values ranged from − 0.05 (WRN) to 0.32 (BARO). Fis values significantly different from zero was found for 23 out of the 31 wild boar populations (Table 1). *Fst- and Jost's D* values are presented in Supplementary Table 3.

The correlation between allele number and sample size followed a logarithmic distribution with more or less pronounced deviations in individual areas (Fig. 2). The highest allelic diversity occurred in the eastern areas, where all sampled individuals were infected with ASF. The deviation from the expected number of alleles (sum of alleles of all markers) based on the sample size and the regression from Fig. 2 is shown as allele excess. Unexpectedly an allele excess was found especially in the ASF positive populations (Supplementary Fig. 1). The lowest allelic excess, on the other hand, was found in the populations of Mecklenburg-Western-Pomerania.

The results of the non-spatial (STRUCTURE) and spatial (TESS, BAPS) algorithms showed a high degree of agreement. Six clusters from STRUCTURE were differentiated in 3 levels. At the first level, STRUCTURE resulted in two clusters (Fig. 3A). Wild boars from the north belonged mainly to cluster 1 (orange), those from the south belonged mainly to cluster 2 (blue) and wild boars along the Elbe valley belonged to both clusters in similar proportions. A separate STRUCTURE run was performed for each cluster at the first level. This further split cluster 1 into 3 sub-clusters, with cluster 1.1 being split again in the third level run. The run for cluster 2 showed 2 sub-clusters.

This resulted in a significant differentiation along a line starting between Magdeburg (MDS) and Halle (HAL) in the west and running via Berlin (B) and the A11 freeway to the north-east (Fig. 3A and D). This line represented the strongest barrier within the study area. The north-western part was clearly sub-structured, but the extreme north-east of the study area was particularly uniform (WRN, PW, NZ, UM, BARW). To the west, there was some differentiation with these areas and LUP (north and south of the A24 freeway) and a significantly marked differentiation with the Southern Heath in Lower Saxony (HK). A second differentiated area existed in the Middle Elbe Plain area. The differentiating effect of the A24 freeway between LUPN and LUPS was significant. The populations were also significantly differentiated between the SDL in the Elbe plain and the more northern areas. On the other hand, lower levels of differentiation between LUP and BARW argue for an existing connectivity along the northern bank of the A24 freeway.

The westernmost area HK in Lower Saxony was more strongly associated with the Middle Elbe Plains than with the LUP. The entire Elbe region showed a high degree of connectivity. There was a gradual exchange between the northern areas (LUP), the south, and even the south-east. The strongest local differentiation was due to the A11 freeway in the north-east of Berlin (see BARW vs. BARO). BARO showed connectivity with MOL in TESS. FF, OS and LOS differed from the other areas, with one cluster appearing preferentially here (cluster 3 in BAPS and cluster 4 in TESS, respectively). HAL and C in the southwest could also be distinguished from the remaining southeastern areas in TESS. STRUCTURE confirmed the differentiation of the southwestern areas, but revealed a connectivity along

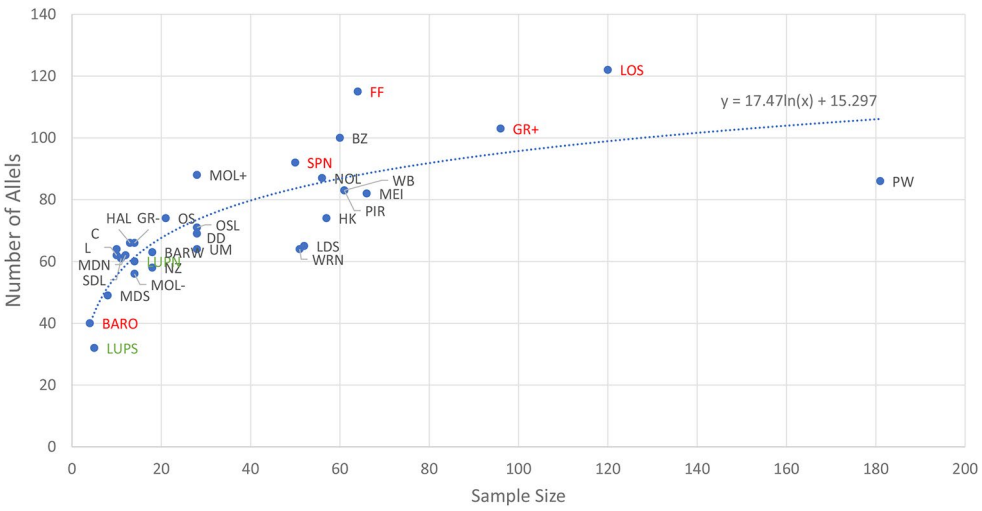

Fig. 2 Allelic diversity as a function of sample size. Alleles and sample size follow a logarithmic relationship. Areas below the trend line have fewer alleles than expected based on sample size, and vice versa. Dotted line: trend line with formula; red font: Populations with exclusively ASF-positive samples; orange font: Areas with a low proportion of ASF-positive animals that could not be evaluated separately

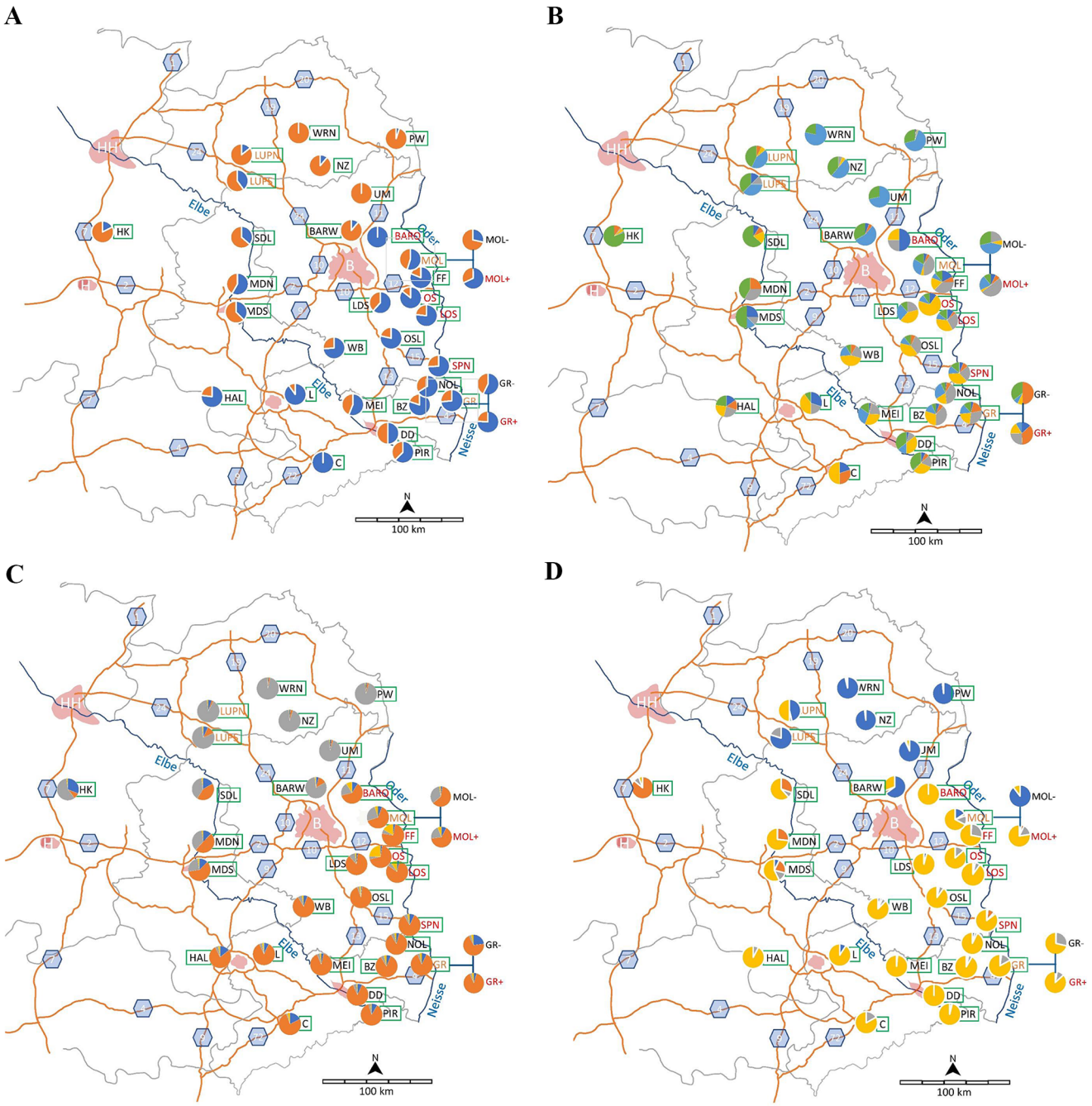

Fig. 3 Distribution of genetic clusters, showing population structuring in wild boar populations in East Germany in 2020–2022. In panels A-D, we used the individual cluster assignment probabilities resulting from Bayesian approaches to visualise the cluster shares for the populations. The cluster assignment probabilities (%) of individuals were averaged for each population. Different colours in the pie charts represent different clusters. (**A**) Results of the first step of STRUCTURE. (**B**) Results of the hierarchical STRUCTURE analysis. (**C**) Distribution of the 4 wild boar clusters after TESS analysis (K = 4). (**D**) Distribution of the 4 clusters after BAPS analysis (K = 4). The population are coded like in Fig. 1

the Elbe valley up to the Elbe Sandstone Mountains (PIR) near the Czech Republic. Differentiation of areas in the east was difficult with STRUCTURE. There was also little directional differentiation in the east using BAPS. Cluster 3 in STRUCTURE appeared in the very eastern populations.

Within the MOL and GR areas, it was possible to compare ASF-positive and ASF-negative wild boars. In both areas they were clearly differentiated from each other. In addition, animals from MOL, including ASF positive and

negative animals, were significantly differentiated from ASF positive and negative animals from GR.

By merging the results from STRUCTURE, TESS and BAPS, the aspects described above were further strengthened (Fig. 4, Supplementary Table 4). The resulting 9 clusters were used to quantify the differentiation between the populations (sum of individual cluster assignment probabilities between the populations), where in pairwise comparison 0 is given if both have the same assignment probability in all clusters, 100 if the assignment probabilities are absolutely different in all clusters (Fig. 5). This illustrates the barrier effect of the A11 freeway, Berlin, a region west of Berlin, and a region between Berlin and Hamburg (Fig. 5). The highest connectivity was found in the Mecklenburg Lake Plateau with Hinterland and Uckermark in the northeast and the Spreewald, Lower Lusatian Heath and Upper Lusatian Plateau in the south-east. Further connectivity could also be inferred for the southeast/east. BARO was clearly differentiated from all areas further south/southeast. The same was true for FF north of the A12 freeway. The differentiation of the local populations between Berlin and the Oder river to the ASF negative animals in MOL (MOL-) was clearer than that to the ASF positive animals (MOL+). South of the A12 freeway, however, there was a high degree of connectivity. The populations were practically all no longer significantly different. Exceptions were animals from MOL and GR which were differentiated. This high degree of connectivity extended westwards to LDS, WB and MEI and southwards to DD and PIR.

Genetic distances between populations (sum of individual cluster assignment probabilities between populations; Supplementary Table 4) were used to statistically test for barrier effects. Genetic distances of populations separated by the barrier were compared with those of populations on the same side of the barrier by analysis of variance. The results showed highly significant results for the A11 freeway and the A24/Elbe Plain and significant results for the A9 and A12 (Table 2). A potential barrier effect of the A15 freeway could not be demonstrated. The barriers explained 43% ($P < 0.001$) of the genetic variance of the populations' distances in the A11 area, 27% ($P < 0.001$) of the variance in the A24/Elbe Plain area. The A11 area increased the genetic distance between populations on different sides of the barrier by 70%, the A24/Elbe Plain area by 67%, the A12

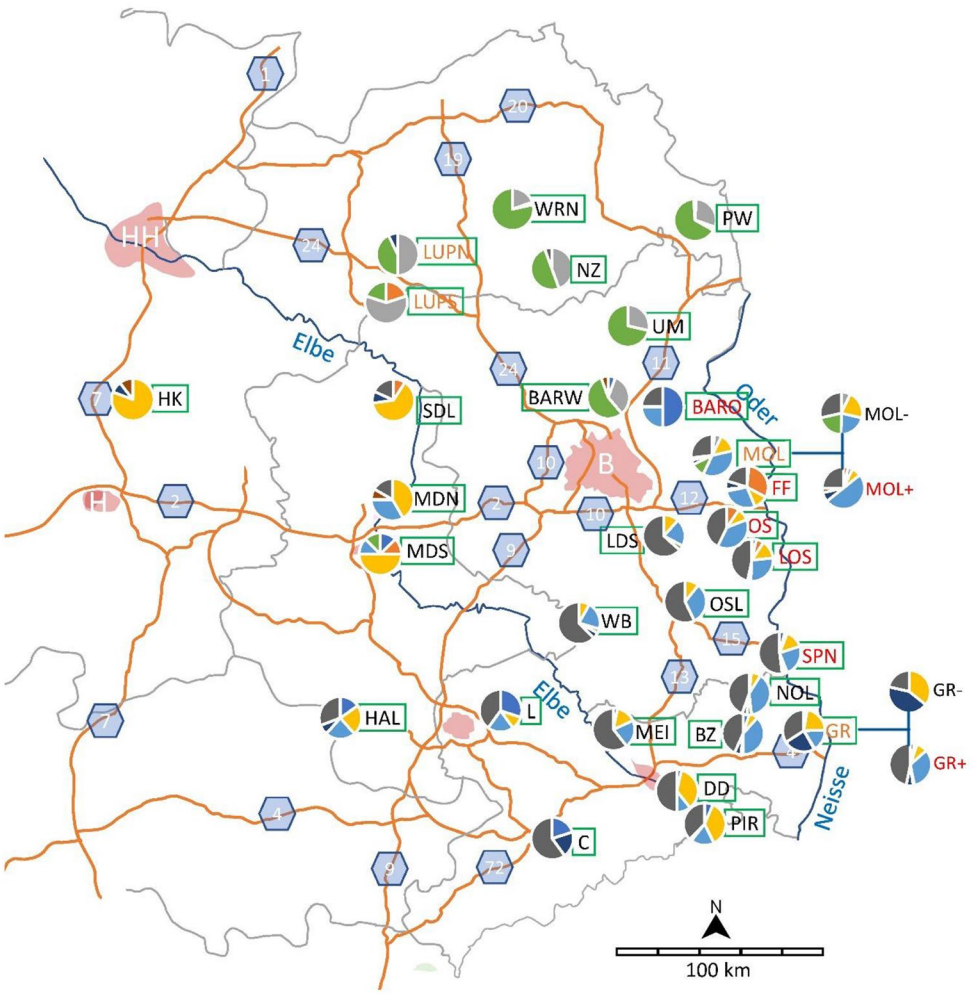

Fig. 4 Distribution of genetic clusters obtained from merging the results of the three separate cluster analyses. Cluster assignments in STRUCTURE, TESS and BAPS of 1800 individuals were simultaneously re-clustered (K = 9). The proportions of the nine clusters are shown in pie charts. Different colours represent different clusters. The populations are labelled with letters according to Fig. 1

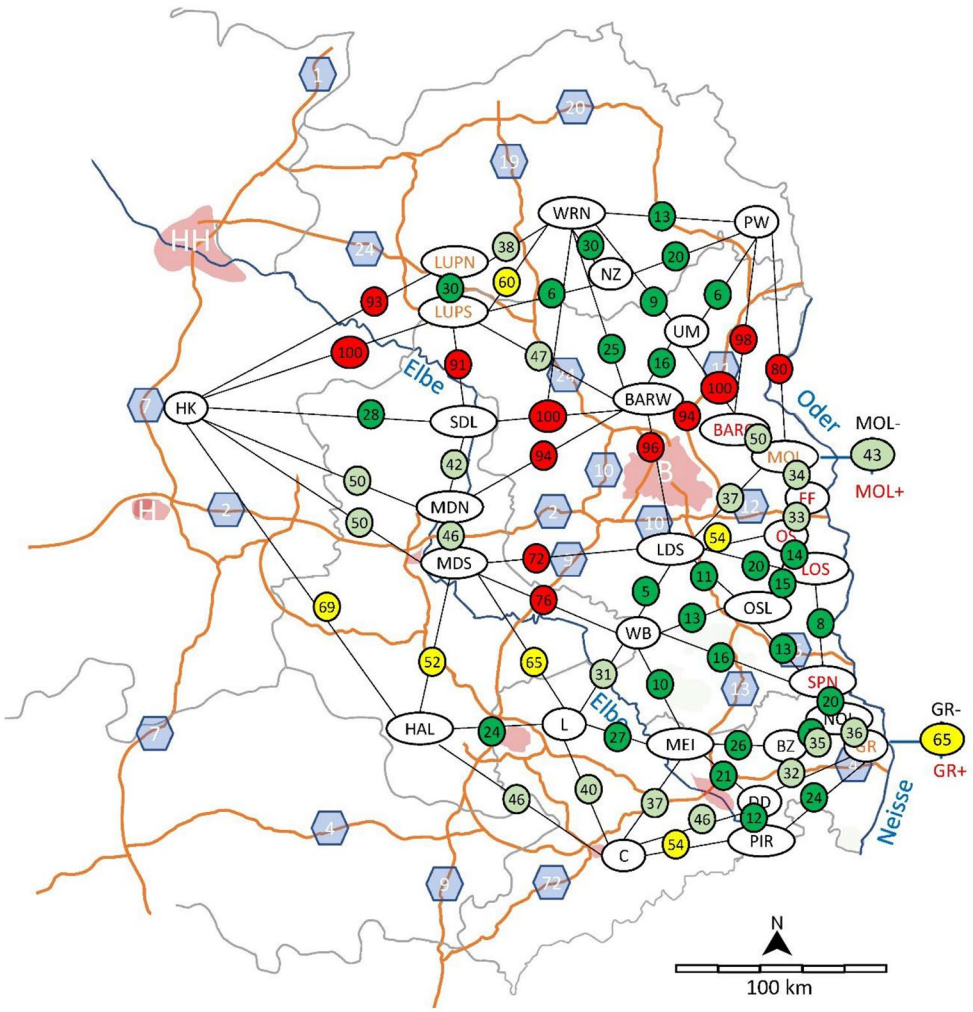

Fig. 5 Pairwise quantification of genetic differentiation of neighbouring wild boar populations in East Germany in 2020/2022. The numbers between two populations represent the distance between the two populations. 0: no deviation, exactly the same distribution; 100: the individuals of the two populations are assigned to completely different clusters. The corresponding pairwise distances for all areas are shown in Supplementary Table 4

Table 2 Effects of distinct barriers and geographical distance on genetic distances (sum of deviations in individual cluster assignment probabilities) between populations after synchronous clustering of TESS, BAPS and STRUCTURE results

	A9	A11	A12	A15	A24/Elbe
Genetic distance same side	42.1	23.0	26.6	18.0	27.0
Genetic distance crossing area	56.1	77.4	38.1	15.8	81.3
R^2 Crossing (%)	5.6	43.4	9.2	2.8	27.4
R^2 Distance in km (%)	28.7	2.2	6.3	4.1	2.2
Δ Genetic differentiation by barrier (%)	25.0	70.3	43.2	-13.9	66.8
P Crossing area	<0.05	3.2×10^{-7}	<0.05	>0.05	8×10^{-5}

Same side: Average genetic distances (sum of deviations in individual cluster assignment probabilities after joint cluster analysis) between populations situated at the same side of a barrier; Crossing area: average genetic distances of populations separated by the barrier; R^2 Crossing: Coefficient of determination, i.e. the percentage of the total variance in the genetic distance explained by the barrier (in %); R^2 Distance in km: Coefficient of determination of the total variance explained by the geographical distance (in %). Δ: Increase in genetic differentiation by barrier (%); P: significance

area by 43% and the A9 area by 25%. A significant barrier effect was also found for the region around the A9 freeway ($P<0.05$), albeit at a lower level.

To emphasize the differentiation between the populations, the genotypes of the ASF viruses in the ASF-positive wild boars were also included in addition to the genotypes of the wild boars. Among the 389 ASF viruses isolated, 8 variants were detected (Fig. 6). Two different variants occurred in the LOS, OS and SPN regions. Only one ASF variant was detected in the other ASF-positive areas. In LOS, variants I.1 and II.1 were found, while in OS, variants II.1 and III.2 occurred simultaneously. In SPN, variants IV.1 and IV.2 cooccurred. Infected animals in LUPN and LUPS

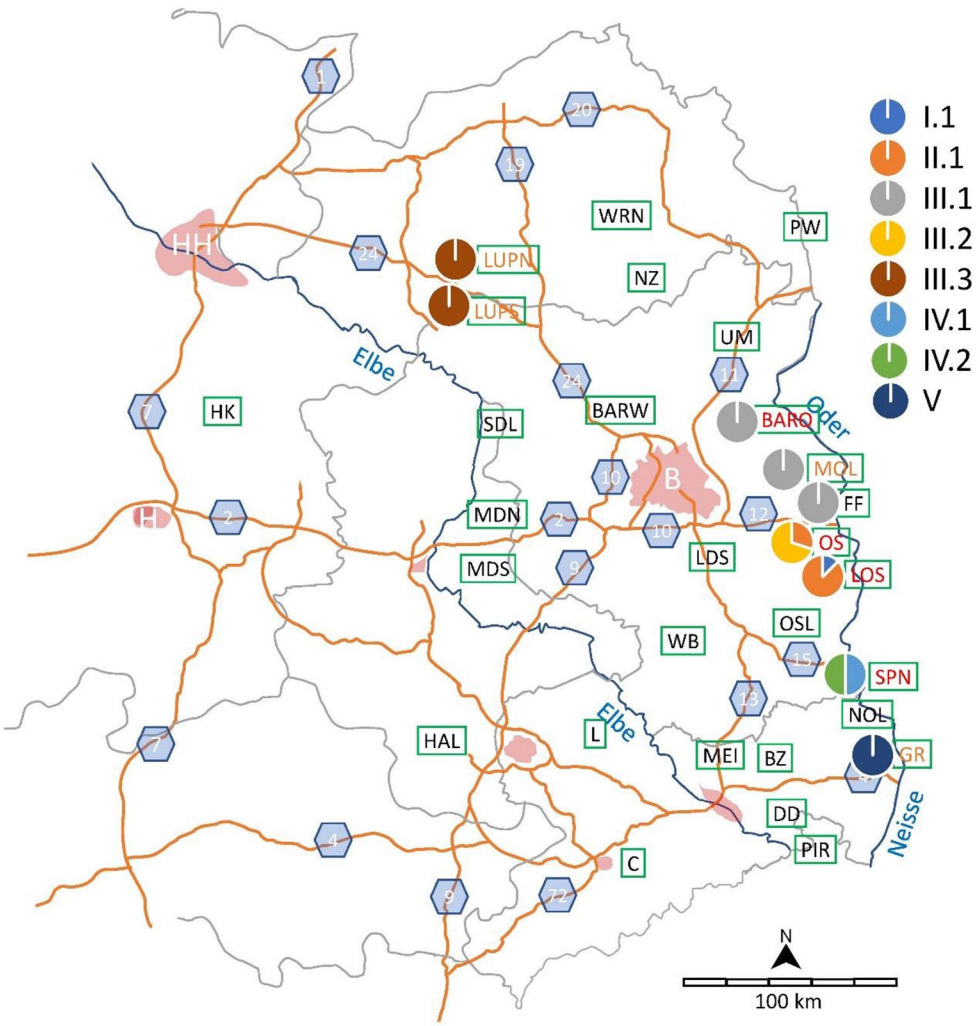

Fig. 6 Distribution of different ASFV variants in 2020/2021 in the study area

carried variant III.3, infected animals in BARO, MOL and FF carried variant III.1.

The results of individual-based Bayesian clustering may be biased by an isolation by distance pattern of the data. A Mantel test (Supplementary Fig. 2A) indicated that this pattern of genetic divergence was significantly predicted by distance (Mantel $r = 0.473$, $P = 0.0001$), such that approximately 41% of the genetic divergence across the study area was explained by geographic distance. To assess the spatial relationship between genetic and geographic distances, we performed a Mantel correlogram analysis. The Mantel correlogram (Supplementary Fig. 2B) included 10 distance classes and showed an almost linear decrease in Mantel r with increasing geographic distance. There was a positive spatial autocorrelation in genetic distance between localities ≤ 175 km, which can be regarded as the size of the genetic patch where individuals have greater genetic similarity than those separated by > 175 km (negative spatial autocorrelation).

Discussion

A current epidemic of African swine fever (ASF) poses a significant threat to wild boar and domestic pigs, particularly in the EU, Eastern Europe and Asia (Sauter-Louis et al. 2022). Due to the great epidemiological importance of wild boar in the spread of the virus and the development of enzootia in affected regions, wild boar management must take into account the dynamics of the spread of this species in the target region. Therefore, the aim of the present study was to provide information on the connectivity of wild boar populations in a region (eastern Germany) with high wild boar density and recent ASF introduction. Based on the genetic differentiation between neighbouring populations, the aim was to determine long-term effective connectivity between these populations and thus characterize the entire region.

Indeed, the chosen methodology revealed considerable regional differences in connectivity between populations. Large uniform areas of low genetic differentiation between populations were found for the entire state of

Mecklenburg-West Pomerania, covering an area of approximately 23,000 km^2. This area was clearly separated from the southern wild boar populations by an area between the A24 freeway and the Elbe Plain to the south-west, by the city state of Berlin to the south and by a region around the A11 freeway to the north-east. A second region of low genetic differentiation was found in the whole south-eastern part of the study area with the Oder and Neisse rivers as the eastern border and the Elbe as the western border. Unfortunately, due to lack of samples from Poland, it was not possible to directly analyse the genetic differentiation between East German and Polish populations. However, it was noticeable that the ASF-positive subpopulations along the German-Polish border showed some distinct characteristics, e.g. cluster 3 (blue) in BAPS analysis and cluster 2 (red) in the merged cluster analysis, suggesting that these clusters might be more common in Poland. However, further studies are needed to substantiate this suspicion. These results were expected, as ASFV is thought to have been introduced from Poland via wild boars (Forth et al. 2023).

Although the quantification methodology was optimised and slightly modified compared to the work of Reiner et al. (2021), the effects of the main barriers in this study are comparable to the enormous effect of the Rhine river in Rhineland-Palatinate (Goedbloed et al. 2013; Reiner et al. 2021). The sampling density in some areas was not sufficient to unambiguously assign the barrier effect to closely neighbouring landscape structures (e.g. river Elbe vs. freeway A24). The potential of main rivers to restrict, although not completely prevent, dispersal of the wild boar has been described previously (Ferreira et al. 2009; Tadano et al. 2016; Reiner et al. 2021). Compared to the discharge of the Rhine of about 2300 m^3/s (January 2024; https://undine.bafg.de/index.html) and the Moselle (about 320 m^3/s), the discharge of the Elbe is about 700 m^3/s and that of the Oder about 540 m^3/s. These values are subject to strong fluctuations depending on the general weather conditions and water levels. According to Reiner et al. (2021), who found no effect of the Moselle but a clear effect of the Rhine, neither the Elbe, nor the Oder can be interpreted as a barrier for wild boar. Experiences from the ASF areas on the Oder and Neisse rivers confirm the free passage of numerous wild boars in both rivers. Furthermore, the natural areas of the Elbe, Oder and Neisse rivers are ideal habitats for wild boars. The Lupin South population (LUPS), located between the A24 freeway and the Elbe, showed a high degree of similarity to LUPN north of the A24, but a significant genetic differentiation from SDL on the opposite side of the Elbe. This finding suggests that the Elbe plain may have a greater differentiating effect here than the A24 freeway. However, this question cannot be answered conclusively in this study due to the small number of populations sampled in this region and the associated low resolution. Thus, it was not possible to determine what the barrier between LUPN and LUPS is in the area of the Elbe valley.

Depending on the local situation, significant (Tadano et al. 2016; Lecis et al. 2022) or only minor (Franz et al. 2012; Reiner et al. 2021) effects of freeways on the genetic differentiation of wild boar have been found. The present study found differentiating effects of the freeways A11, A9, A12 and, at least partially, A24, but no effect of the freeways A15 and A20. There were not enough sampling points to investigate the effects of the other freeways. It is thought that, unlike other large game species, wild boar can easily cross freeways, both by overcoming fences and by using smaller ditches and pipes under the roads. In general, the effects of freeways, particularly on wild boar, cannot be separated from those of their immediate surroundings. In summary, it is difficult to make a general statement about freeways, as the specific situation of the individual freeway and its surroundings must be considered.

In contrast to the situation across the Elbe plain, there was little differentiation along the Elbe, at least from the Lower to the Middle Elbe plain. As this region is sparsely populated and largely designated as a nature reserve, with roads and freeways crossing large bridges, there appears to be a good exchange between the wild boar populations over large areas.

The very different levels of connectivity between the wild boar populations in the study areas, as determined by the genetic differentiation between the populations, could influence the spread of ASF in different ways.

The first outbreak in the study area occurred in September 2020 in the district of Spree Neisse (SPN) on the border with the district of Oder Spree (LOS). At the end of September, the second outbreak occurred in Märkisch Oderland (MOL) (FLI 2020). There was a strong connectivity between the wild boar populations in the whole region around SPN, so that a spread to the neighbouring areas LOS, OSL, NOL and even up to WB seemed possible. Differentiation from other wild boar populations only increased from the A4 freeway to the south and the A12 freeway to the north. Significant differences between SPN and MOL were detected at different population genetic levels and were also highlighted by Bayesian clustering methods. They suggest that the second outbreak in MOL was not a gradual spread via wild boar, but a new entry from Poland. This hypothesis is ultimately supported by the genetic characterisation of the ASFV variants. The first outbreak in SPN was variant IV, while the outbreak in MOL was variant III. Neither variant overlapped in any of the areas (although all ASF-positive wild boars were tested) and the wild boar populations between these areas remained free of ASF. The assumption that the two outbreaks occurred independently of each other via the

Oder River from the east is also supported by the short distance of the respective areas from infected populations on the eastern side of the Oder river and the special ecological importance of the Oder River as a mixing area for wildlife (Kächele and Dabbert 2002).

Both centres of infection subsequently spread very slowly. By the end of the study, the ASF virus variant had reached the immediate neighbouring populations of MOL, FF in the south (February 2021) and BARO in the northwest, thus remaining within the range of low differentiation between the affected populations. In both cases, there is also the possibility that the infection did not originate in MOL, but came directly across the Oder River from Poland. Striking differences in the cluster composition of ASF-positive and ASF-negative wild boars in the MOL area underline this possibility. Information on the genetics of wild boars on the other side of the Oder may help to clarify this issue. In any case, the virus variant did not succeed in infecting populations of higher genetic differentiation across the Berlin, A11 and A12 barriers. Despite the low genetic differentiation between SPN and the neighbouring populations, virus variant IV was also unable to spread further due to the management measures applied in the area (e.g. area closure, fencing, search and removal of wild boar carcasses).

In January 2021, wild boars were infected near Görlitz (GR) (FLI 2021). The connectivity to already infected populations further north was found to be good with low genetic differentiation. However, close proximity to infected populations east of the Neisse River and a new virus variant (V) were detected (FLI 2023), suggesting that infections were introduced from Poland again. ASF-positive and ASF-negative wild boars in the GR area showed significant genetic differentiation, further suggesting that the ASF-positive wild boars may be dispersal from Poland. This is also supported by the concentrated occurrence of private alleles in the ASF-positive animals.

During the summer and autumn of 2021, the disease spread further north, always along the Oder River. Outbreaks west of the Oder were always preceded by new outbreaks east of the Oder. In December 2021, a population in the area of Ludwigslust (LUP) was infected, a long way from the ASF-positive regions in the east. As only a few animals were affected north (LUPN) and south (LUPS) of the A24 freeway, it was not possible to distinguish between the two groups. Direct transmission from BARO, the next infected area in the south-east, is rather unlikely due to the geographical distance, the jumping spread and the high barrier function of the area between the two regions, especially because of the A11 freeway. Once again, it is the exclusive occurrence of a new virus variant (III.3) that proves a new entry independent of already infected wild boar populations in Germany. The new variant clearly underlines the assumption that infection did not occur via wild boar but via humans as mechanical vectors. In fact, the LUP region north and south of the A24 freeway was infected with the same virus variant, but this variant could not be matched to any of the variants of the other infected populations. It remains unclear whether the wild boars on both sides of the A24 freeway were infected by humans or whether the infection could have spread to the other side of the freeway via infected wild boar. Very similar genotypes were present on both sides of the freeway.

The overall picture is one of multiple introductions of different ASFV variants from Poland along the Oder and Neisse rivers. The infected areas are then enzootic on the German side with a low tendency to spread. Sudden jumps over long distances seem to be possible at any time, independent of a direct involvement of wild boars. Genetic differentiation seems to be well suited to confirm or reject the direct role of wild boars in more distant outbreaks. The more pronounced barriers identified by genetic analysis have not been overcome by the disease in the last three years. The local spread is also broadly consistent with the calculated connectivity. However, the use of genetic information on the virus variants is crucial for assessing the spread of the virus.

The assumption that the genetic differentiation of populations at least partly reflects the long-term gene flow between them (Reiner et al. 2021) and that clustering algorithms can be used in a spatial context, as outlined by Safner et al. (2011) and Reiner et al. (2021), is supported by the present study. Due to differences in efficiency and reliability in detecting genetic boundaries in different populations and landscapes and with different markers (Safner et al. 2011; Basto et al. 2016), we used three different Bayesian algorithms implemented in the non-spatial STRUCTURE and the spatial BAPS and TESS analysis programs, and finally merged the results in order to identify general effects and avoid overestimating more random aspects of individual analyses. Areas of barrier function and regions with no restriction of genetic exchange between populations were identified.

However, the sub-structuring of wild boar populations in eastern Germany may not only be the result of real barriers, but also of gradients in landscape resistance, and most importantly, isolation by distance (Cushman et al. 2006). Bayesian clustering methods generally tend to overestimate the number of clusters in the presence of isolation by distance (Frantz et al. 2009; Safner et al. 2011). Structuring of genetic diversity due to isolation by distance at the largest scale can be detected by the Mantel test. Mantel tests within distance classes showed a positive spatial autocorrelation up to about 175 km, indicating that evolutionary processes are dominated by gene flow up to this distance. Given the

spatial extent of the study area (north-south extent = 450 km; west-east extent = 250 km), strong isolation by distance patterns within and between neighbouring regions cannot be expected. Therefore, the results of the Bayesian clustering methods should be valid. The identified threshold of 175 km is much higher than that of 100 km in the Rhineland-Palatinate study (Reiner et al. 2021). The difference corresponds to the smaller landscapes in Rhineland-Palatinate.

Another key question regarding the rationale of this study is the need for absolute barriers to contain epidemics. The actual relationship between the degree of genetic differentiation and the limiting effect on the spread of ASF remains unclear. In principle, the local situation of virus spread will depend on individual effects, the current situation of climate, weather, food supply, social structure, hunting pressure and other factors that cannot be predicted with the applied method (Reiner et al. 2021). However, given the limited contagiousness of the virus, it can be assumed that the stronger barriers identified in the present study and by others (Reiner et al. 2021) should provide a usable barrier under normal overriding pressure from wild boar, at least compared to the absolutely barrier-free areas of the Mecklenburg Lake District with Hinterland and Uckermark, along the Oder and Neisse rivers, Spreewald (OS, OSL, SPN) and Lusatia. Direct animal to animal transmission plays the most important role in infected wild boar populations. Wild boar contacts are particularly frequent within groups, but also between groups, as their territories often overlap. Lack of food and cover, supplementary feeding and hunting pressure increase the size of home ranges and the likelihood of contact (Johann et al. 2020a, b). Young animals aged 0.5 to 2 years are disproportionately involved in intergroup contacts, particularly for the biological purpose of reproduction (Podgorski et al. 2018). Targeted culling of yearlings is therefore particularly important to reduce the risk of infection.

Other key factors that promote the enzootic nature of the ASF are the long-term availability of infectious material in carcasses due to high virulence (large amounts of virus) and pronounced persistence (Chenais et al. 2018; Pikalo et al. 2020) and the fact that animals in an affected region are only sporadically infected due to low contagiousness, meaning that the source of the disease persists for a long time. Resilient animals (Eblé et al. 2019; Ståhl et al. 2019) also allow the disease to spread to other regions over time. Infected animals can transmit the virus for up to 70 days, and the possibility of virus shedding persists for up to 100 days in wild boar (Blome et al. 2020). At the same time, transmission by vectors (i.e., soft ticks [*Ornithodoros*]) does not appear to be important in the current European outbreak (Costard et al. 2013). The role of other arthropods also appears to be limited in the spread of disease across areas (Blome et al. 2020). Therefore, controlling the movement of infected animals is at the heart of the EU's prevention and control of ASF (EFSA 2018). This involves reducing hunting pressure in the newly infected area, but greatly increasing it in the surrounding area, together with the creation of barriers (especially fences) to keep infected animals in as small a range as possible. This approach has repeatedly limited or even stopped the spread of ASF, as in Belgium and the Czech Republic (Dixon et al. 2020). However, fences can have significant landscape and conservation impacts, as well as significant direct and indirect (genetic isolation) losses to other wildlife species. This is particularly important if disease eradication is not possible in the short term. The results of the current study should help to strengthen existing barriers locally, for example by closing passages under freeways such as the A11, which are already effective barriers, rather than erecting large-scale fences elsewhere. In addition, fencing does not appear to be very effective in areas where wild boar has unrestricted access. The results from Rhineland-Palatinate also show potential barriers for wild boar across the state, while natural barriers to the spread of ASF in north-eastern Europe (EFSA 2018) were not detected under the then existing conditions of massive infection pressure and simultaneous spread of the virus by wild boar and anthropogenic sources, although these results were considered preliminary and should be interpreted with caution (EFSA 2018).

The results of the present study show that microsatellite based genetic assessment of population similarity can be used to investigate the movement of individuals in a potentially infected population. However, spontaneous small-scale dispersal cannot be predicted.

Compared to other wild animal species, the genetic variation of wild boar in Central Europe is considered to be relatively low (Vilaça et al. 2014). In addition, wild boar is considered to be less restricted by barriers than many other mammals (e.g., red deer, *Cervus elaphus*; Vassant et al. 1993; Tottewitz et al. 2010; Dobias and Gleich 2010; Frantz et al. 2012) and genetic differentiation could be hindered by the large number of wild boars in the study area, which is likely to counteract genetic drift due to large effective population sizes (Frantz et al. 2012).

Conclusions

In the study area, a distinct barrier for wild boar was identified from the lower and middle reaches of the Elbe through Berlin and the A11 freeway to the Polish border. This contrasted with the largely barrier-free north and south-east. The introduction of ASF from Poland occurred in several waves via the Oder and Neisse rivers. The outbreaks remained

within the boundaries of populations that were expected to be more closely linked due to low genetic differentiation. If more distant populations with higher genetic differentiation were infected, natural wild boar contacts could be excluded as a direct vector. The hypothesis of virus introductions along the Oder/Neisse rivers and to more distant populations was supported by genetic characterisation of the virus variants. The combination of host and virus genetic analyses can be recommended to other affected regions to support their ASF management. The effect of potential barriers, such as freeways and rivers, in preventing the dispersal of wild boars and thus the spread of the virus does not follow general rules and should be investigated on a case-by-case basis.

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Author contributions Author contribution Planning of the study GR SBe S Bl; Sample collection and DNA analyses US, VF, CS; Elaboration of the virus variants FJH; Support of the laboratory work KG, HW, SBl; Population genetic analyses US, HW; Support of the work, evaluation and writing of the manuscript GR; All authors reviewed the manuscript and assisted in its preparation. All authors reviewed the manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no competing interests.

Ethical approval No animal experiments were performed in the present study. Only samples of wild boar already collected before the study, by authorised persons during legalised hunting within the framework of wild boar management and ASFV monitoring, were used.
No animals were killed specifically for the study. No living animals were sampled.

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