Belgian Journal of Zoology
www.belgianjournalzoology.be

ISSN 2295-0451

Research article

https://doi.org/10.26496/bjz.2024.181

Origin of the invasive North American beaver (*Castor canadensis*) sampled in Western Europe

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Abstract. The North American beaver (*Castor canadensis*) has been present in Western Europe since 2006. Its occurrence has been recorded in Luxembourg, Rhineland-Palatinate (Germany) and Wallonia (Belgium). As *Castor canadensis* is a direct competitor of the Eurasian beaver, it is classified as an invasive species due to ecological niche overlap, with a notably higher reproduction rate. Its colonization is therefore a significant threat for native Eurasian beavers and its populations should be eradicated. The origin of the North American beaver populations in Western Europe is also still unknown. The present study aimed at investigating the genetic structure and the putative origin of *C. canadensis* individuals collected in Western Europe. To achieve this goal, we compared their genetic characteristics with those of individuals coming from Finland, the USA and from the German zoo of Lünebach (Eifel). Our results revealed that all the individuals sampled in Western Europe show close relationships and belong to the same genetic cluster. Given their genetic link to the Eifel zoo beavers, the hypothesis of this zoo being the origin of wild *C. canadensis* populations in Western Europe seems the most probable. As no North American beavers have been detected in France, we can also conclude that the eradication measures implemented in Belgium, Luxembourg and Germany since 2006 seem to have been relatively efficient, thus preventing the spread of this species to other countries. However, future monitoring still has to be performed in order to confirm the total eradication of this invasive species in Western Europe.

Keywords. North American beaver, invasive species, genetics, population structure, microsatellites, Eurasian beaver.

Pigneur L.-M., Gailly D., Manet B., Herr J., Schley L., Venske S., Bressan Y. & Michaux J. (2024). Origin of the invasive North American beaver (*Castor canadensis*) sampled in Western Europe. *Belgian Journal of Zoology* 154: 83–95. https://doi.org/10.26496/bjz.2024.181

Introduction

The North American beaver (*Castor canadensis* Kuhl, 1820) is native to Canada, the United States of America (USA) and northern Mexico (see Rosell & Campbell-Palmer 2022). Nevertheless, it was introduced into Finland around 1937, after the massive decline of the Eurasian beaver *C. fiber* Linnaeus, 1758 in the previous centuries; however, at the time of those introductions, it was not known that North American and Eurasian beavers were two separate species (Lahti & Helminen 1974; Parker *et al*. 2012). These two species are also difficult to identify based on morphological traits and DNA analysis is needed to distinguish them without any ambiguities even though they are two different species, that cannot hybridize, having been separated for a long time. The North American population introduced in Finland later spread into Karelia (Russia) (Danilov & Kan'shiev 1983).

Additionally, a recent center of presence of this species was reported in Western Europe: since 2006, the occurrence of the North American beaver has been recorded in several locations in Luxembourg, Rhineland-Palatinate (Germany) and Wallonia (Belgium) (Frosch *et al*. 2014). As the species is a direct competitor of the Eurasian beaver due to ecological niche overlap, it is classified as an invasive species. With a notably higher reproduction rate, its presence is a significant threat for the native Eurasian beaver (Nummi 2001; Parker *et al.* 2012; Petrosyan *et al.* 2019). Therefore, local North American beaver populations should be eradicated (Nolet 1997). Knowing the origin of populations of the North American beaver is important from a conservation point of view in order to avoid further escapes or intentional releases.

However, the origin of the aforementioned North American beaver population in Western Europe is still unknown. Various hypotheses have been suggested: in 1965, a reproduction center was established in Neustadt an der Donau (Bavaria, Germany), with beavers from USSR, Poland, Sweden and Finland (Smit & van Wijngaarden 1981). This center thus potentially included *C. canadensis* individuals (from Finland or Russia). It was reported that several individuals escaped from this center (Véron 1992), and this could be the origin of the colonization in Western Europe. *Castor canadensis* could also have been introduced in Bavaria in the 1970s, when beavers from breeding centers in Poland and Russian were used for a reintroduction project (Nolet & Rosell 1998). Finally, a third putative source of the presence of *C. canadensis* in Western Europe is the German zoo of Lünebach (Eifel zoo), located close to the border with Belgium and Luxemburg. Indeed, North American beavers were held by this zoo until 2009. As a number of North American beaver sites were found to be established on the river Prüm that runs through this zoo, it was speculated that the zoo could be involved in the establishment of the North American beaver in the Prüm Valley. To reduce the risk of escapes of *C. canadensis* from the zoo, the last two North American pairs held in the zoo were replaced by four Eurasian beavers in 2009.

The present study aims at investigating the genetic structure and the putative origin of the *C. canadensis* individuals collected in Western Europe. To achieve this goal, we compared their genetic characteristics with those of North American beavers from other European regions (Belgium, Germany, Luxembourg and Finland) and with samples from the USA. Two samples of *C. canadensis* individuals that were removed from the German zoo of Lünebach were also included in the present study.

TABLE 1

Number and origin of analysed samples.

Country	Number of samples
Belgium	4
Luxembourg	11
Germany	11
Finland	3
USA	7

Material and methods

Sample collection

Hair or tissue samples (Table 1, Appendix 1) were collected in Luxembourg by the *Administration de la nature et des forêts*, and in Belgium by the *Département de l'Etude du Milieu Naturel et Agricole, Wallonia*. Noninvasive hair samples were collected using barbed wire traps without animal handling (Herr & Schley 2009). Tissue samples from Germany were obtained during a sterilization program by the federal state Rhineland-Palatinate with the aim to prevent the spreading of the North American beaver (Dewas *et al*. 2011). Finally, an additional 224 beaver samples (133 tissue samples from dead individuals and 91 hairs collected with barbed wire traps or on living individuals) were collected between 2012 and 2022 in the Grand Est and Hauts-de-France regions (North Eastern France) to check for potential *C. canadensis* presence in France.

Species identification (to distinguish between *C. fiber* and *C. canadensis*) was based on DNA sequencing of the complete mt *cyt b* region (Eugène 2010), except for sample C16, which was analyzed by sequencing of the hypervariable domain I of the mitochondrial control region (Vila *et al*. 1999).

This extensive beaver sampling collection allowed us to identify 26 samples of *C. canadensis* obtained in Western Europe: 11 samples from Germany (including zoo specimens), 11 from Luxemburg and 4 from Wallonia (Belgium) (Figure 1, Table 1, Appendix 1). In addition, three samples from Finland and seven from Illinois, USA were included in the analysis for comparisons (Table 1). No samples from France were included as *C. canadensis* was not found to be present there.

DNA isolation, amplification and genotyping

Genomic DNA was isolated from hair or tissue samples using the DNeasy Blood and Tissue kit (Qiagen) with slight modifications to the manufacturer's protocol.

Multilocus genotypes were obtained by PCR amplifications of 14 autosomal microsatellites (Crawford *et al*. 2008; Frosch *et al*. 2011).

The forward primer of each locus was 5'-end labeled with a fluorescent dye. The following multiplex sets were designed: mix A (Cca13, Cca18, CF31, CF32, CF33, CF44, CF06, Cca5) and mix B (CF05, CF07, Cca4, Cca8, CF19, CF41). PCRs were carried out in 10 µl volumes containing 1 µl of primer mix (containing each 2 µM primer), 5 µl of Multiplex PCR Master Mix (Qiagen) and 1 µl of genomic DNA. All amplifications were performed as follows: 95°C for 15 min followed by 40 cycles (94°C for 30 s, annealing at 57°C for 90 s, extension at 72°C for 60 s) and a final extension step at 60°C for 30 min. PCR

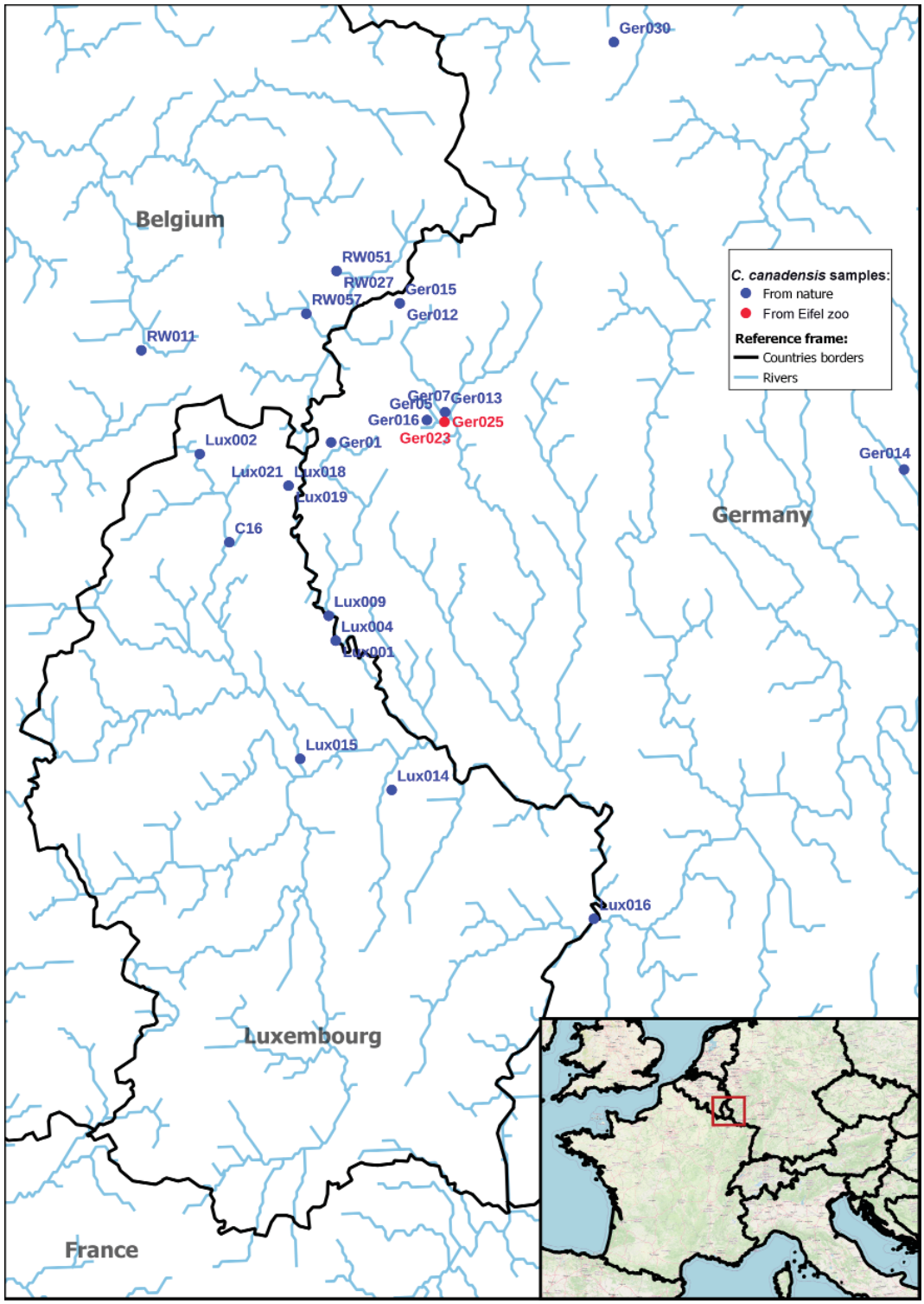

Figure 1 – Sample distribution in Western Europe. Sample codes refer to Table 1. The location of samples collected in the Eifel zoo is represented in red. Map designed under QGIS version 3.22.12 Bialowieza (Quantum GIS Development Team 2013. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org). Cartographic background: HydroRIVERS ©HydroSHEDS (https://www.hydrosheds.org/), ©OpenSteetMap (https://www.openstreetmap.org/), Nomenclature of Territorial Units for Statistics (NUTS) 2016 ©EUROSTAT (http://ec.europa.eu/eurostat/web/gisco/geodata/reference-data/administrative-units-statistical-Units)

products were genotyped on an Applied Biosystems 3730XL Genetic Analyzer at the Université Libre de Bruxelles using 2 µl of amplified DNA, 10 µl of Hi-Di formamide (Applied Biosystems) and 0.15 µl of GeneScan-500 (LIZ) size standard (Applied Biosystems). Length variation determination (alleles and genotypes) was performed using GENEMAPPER 4.0 (Applied Biosystems). MICRO-CHECKER 2.2.3 (Van Oosterhout *et al*. 2004) was used to detect stutter errors and to estimate the proportion of null alleles at each locus for each cluster defined by the STRUCTURE analysis (see below). Genotypes were then corrected accordingly.

Data analysis

The genetic structure of our *C. canadensis* sampling was inferred using Bayesian clustering analysis with STRUCTURE 2.3 software (Pritchard *et al*. 2000). We ran 10 iterations for each *K* value from 1 to 6 using the admixture model. A total of 10^6 MCMC repetitions was performed after a burn-in period of 10^5. STRUCTURE HARVESTER was used to investigate the optimal number of clusters according to the increasing likelihood of the data (Pritchard *et al*. 2000) and the Δ*K* method (Evanno *et al*. 2005). Results of the 10 iterations for each *K* value were summarized and averaged using CLUMPAK (Kopelman *et al*. 2015).

We also performed a Discriminant Analysis of Principal Components (DAPC) (Jombart *et al*. 2010) in *adegenet* version 1.3–8 (Jombart 2008) in R version 2.15.2 (R Development Core Team 2008) to identify and describe clusters of genetically similar individuals. The most likely number of genetic clusters was determined using the k-means clustering algorithm (Legendre & Legendre 1998), for $K = 1$ to $K = 10$, via the function *find.clusters*, with all principal components (PCs). The appropriate number of clusters was defined using the Bayesian Information Criterion (BIC) through the distribution of BIC corresponding to all possible clustering and with the lowest value being generally indicative of the best clustering. The *optim.a.score* function was used to determine the optimal number of PCs and a final DAPC was carried out with this optimal number of PCs.

The genetic relationships among samples was analyzed using Principal Coordinate Analysis (PCoA) as implemented in GenAlex 6.5 (Peakall & Smouse 2012).

Fixation indexes (Fst and Djost) and diversity indexes were calculated with *diveRsity* (Keenan *et al*. 2013) in R both for the genetic clusters determined according STRUCTURE and for the countries (sampling locations).

Results

The STRUCTURE analysis suggests at least 2 genetic clusters within our sampling (highest Δ*K* for $K = 2$; Figure 2). The first cluster includes the individuals from the USA and Finland and the second one gathers the samples from Western Europe (Belgium, Germany and Luxemburg) (Figure 3). Based on mean LnP(D) values, the best *K* would be 3 and this clustering level shows a discrimination between USA and Finnish samples (Figure 3).

The DAPC also suggests three genetic clusters (lowest BIC value) corresponding to the three populations defined above (Western Europe, Finland, USA; Figure 4).

The PCoA shows three main groups with the Finnish and USA samples being well separated from those of Western Europe (Figure 5).

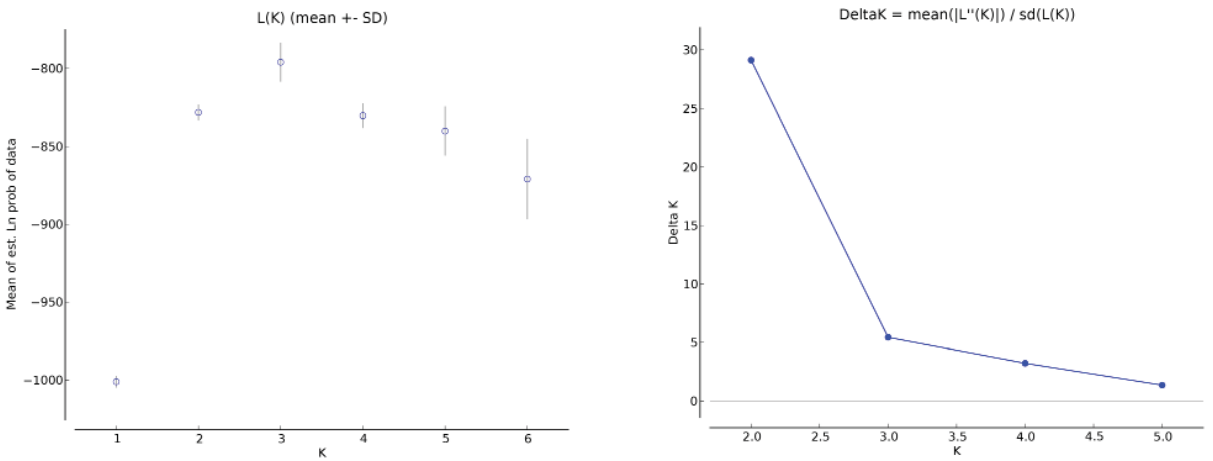

Figure 2 – Results of the Bayesian clustering analysis with STRUCTURE software. Left: mean lnP(D) for each tested K value, right: *ΔK* values for each K.

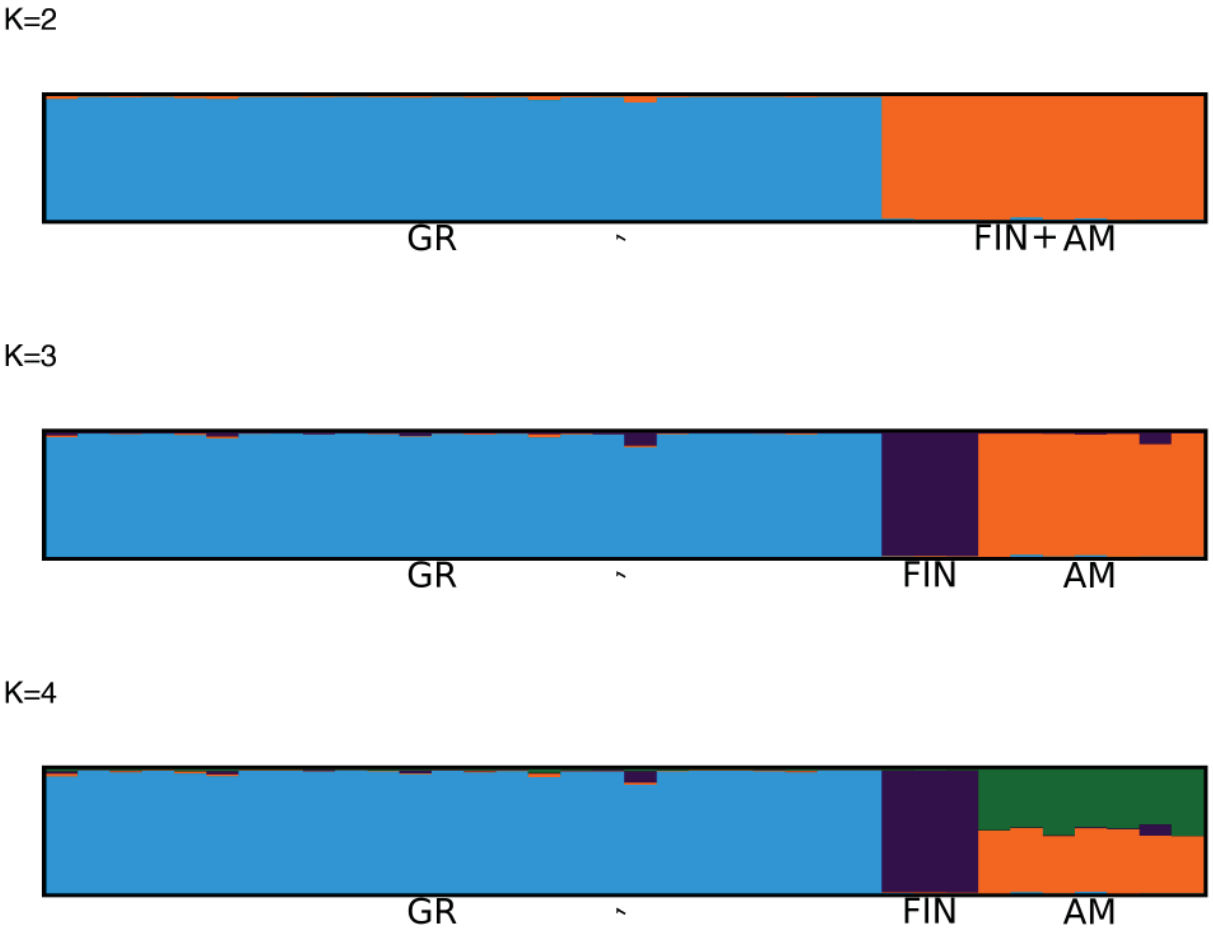

Figure 3 – Genetic structure of *C. canadensis* individuals sampled in Western Europe (GR) (Belgium, Germany, Luxemburg), Finland (FIN) and USA (AM). Barplot of membership coefficient according to STRUCTURE results for K = 2 to K = 4.

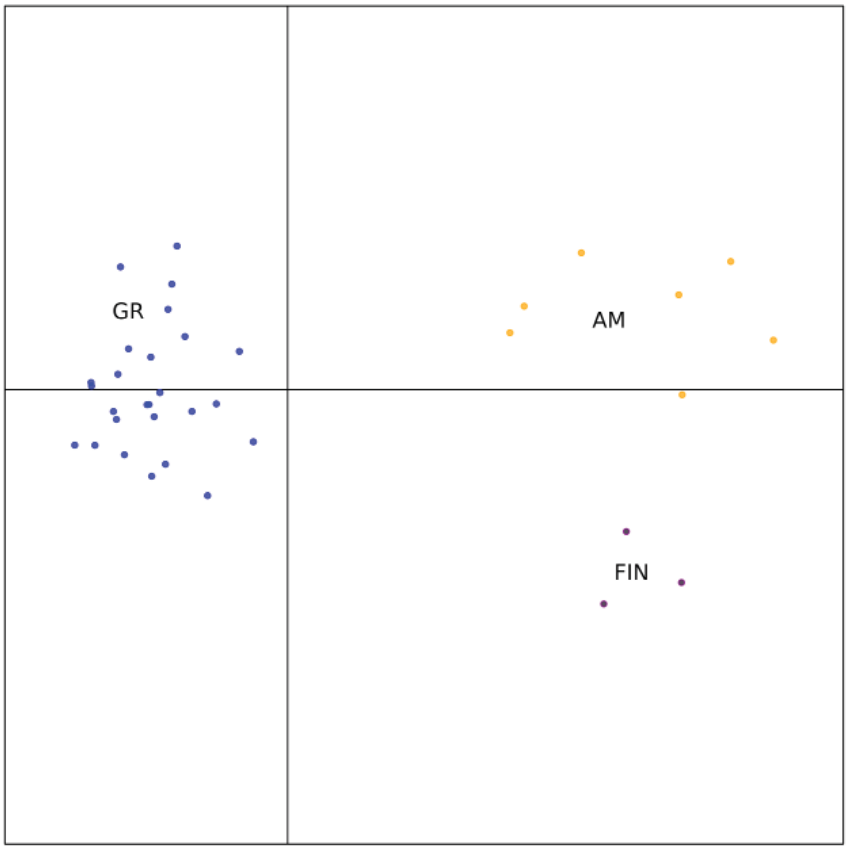

Figure 4 – DAPC plot showing the three genetic clusters. GR = samples from Western Europe, AM = samples from USA, FIN = samples from Finland.

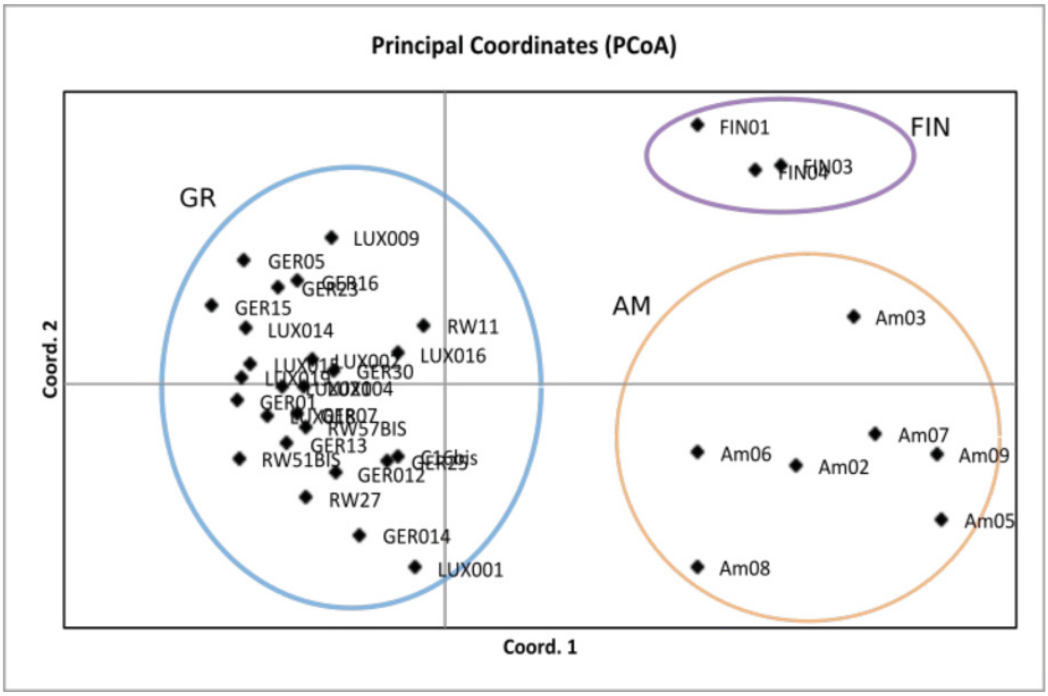

Figure 5 – Two-dimensional PCoA plot based on allelic frequencies. GR = samples from Western Europe, AM = samples from USA, FIN = samples from Finland. Percentage of the diversity distribution explained by axes: axis 1 = 21.71%, axis 2 = 11.14%.

TABLE 2

Fst and Djost estimates (above and below diagonal respectively) between sampling locations (countries). Bel = Belgium; Ger = Germany; Lux = Luxemburg; Fin = Finland; USA = United States.

	Bel	**Ger**	**Lux**	**Fin**	**USA**
Bel	X	0.012	0.0156	0.3698	0.2292
Ger	0.0016	X	-0.023	0.3922	0.2992
Lux	7,00E-04	-0.0011	x	0.393	0.3107
Fin	0.2783	0.2508	0.1897	X	0.22
USA	0.2349	0.2376	0.2329	0.1623	x

TABLE 3

Allelic richness (Ar) within genetic clusters and sampling locations (countries). GR = Western Europe; Bel = Belgium; Ger = Germany; Lux = Luxemburg; Fin = Finland; USA = United States of America.

	Ar
GR cluster	2.4
Am-Fin cluster	3.61
Bel	1.79
Lux	1.74
Ger	1.82
Fin	1.67
USA	2.36

The level of genetic differentiation between the clusters and sampling locations (countries) was assessed using the Fst and Djost estimates. The level of genetic differentiation was very high between the USA-Finnish cluster and the Western European cluster (Fst = 0,2835 ; CI [0,2342-0,3397]). At the level of sampling locations, we observed a pronounced amount of genetic similarity and gene flow between the individuals from Western Europe (Table 2) suggesting that they belong to a same population and they are interbreeding freely. It is unlikely that they directly originate from the Finnish population. Indeed, this latter population seems genetically well isolated from the Western European cluster and there is no gene flow between these groups (Fst > 0.36).

The overall allelic richness seems very low in the beavers of European countries (Western European cluster: 2.4 and Finland alone: 1.67) (Table 3, Appendix 2). However, our sampling was very limited and these indexes might be underestimated.

Within our limited sampling, 67% of the alleles found in wild North American beavers of Western Europe were also found in the two individuals from the Lünebach zoo. Only a few loci harbored alleles that were not recorded in the two genotypes from beavers from the zoo.

All alleles found in these two individuals were also observed in wild *C. canadensis* from Western Europe.

Discussion

The present study aimed to determine the genetic structure and the putative origin of the invasive *Castor canadensis* which has been found in Western Europe since at least 2006.

Our results revealed that all the individuals sampled in Western Europe show close relationships and belong to the same genetic cluster (q > 95%, except one sample). It was not possible to determine direct parentage links due to the limited sampling. It is noteworthy that the individuals that originated from the Eifel zoo (Lünebach) (Ger23 and Ger25) are included in that same cluster and are thus closely related to the North American beavers sampled in the wild in Western Europe. The Eifel zoo, located in Germany near the border to Belgium and Luxemburg, had already been suspected to be at the origin of the *C. canadensis* presence in the Prüm Valley (Dewas *et al*. 2011).

According to our genetic data, it seems unlikely that the Western European population directly derives from the Finnish population highlighted by the lack of gene flow between them. Given their genetic link to the Eifel zoo beavers, the hypothesis of the Eifel zoo as the origin of wild *C. canadensis* populations in Western Europe appears to be the most probable.

The analysis of the two other zoo beavers would have been interesting to complete the present study, particularly in order to test for the presence of certain alleles found in the animals from Western Europe, which did not exist in the two zoo individuals that we were able to analyze. In addition, a more exhaustive sampling of North American populations will be required to precisely determine the provenance of the *C. canadensis* kept at the Eifel zoo, the likely origin of invasive populations in Western Europe.

Finally, as no North American beavers have been detected in North or North-East France, we can conclude that the management measures implemented in Belgium, Luxembourg and Germany since 2006 seem to have been relatively effective, thus preventing the spread of this species to other countries (e.g., France). However, monitoring will still have to be performed in the future, in order to confirm the total eradication of this invasive species in Belgium, Luxembourg and Germany.

Acknowledgements

The authors are deeply indebted to all contributors who provided samples from the different regions, and particularly to Joanne C. Crawford and Heikki Henttonen for providing tissue samples from Illinois and Finland, respectively. We also thank all members of the French Beaver Network and their partners for collecting samples in Northern France. M. Eugène is particularly thanked for her participation in the acquisition of the genetic data. Johan Michaux benefited from FRS-FNRS grants (“directeur de recherches”). The genetic analyses were performed using funding from the Service Public de Wallonie, (DEMNA) and the Administration de la nature et des forêts, Luxembourg.

Data availability

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

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Manuscript received: 24 January 2024
Manuscript accepted: 24 June 2024
Published on: 28 June 2024
Branch editor: Peter Galbusera

Appendix 1

Origin of *C. canadensis* samples used in the present study.

Code	Country	Sampling date	Locality
RW011	Belgium- Wallonia	17/03/10	Gouvy (Bovigny)
RW027	Belgium- Wallonia	25/02/10	Sankt-Vith (Eiterbach site 14)
RW051	Belgium- Wallonia	1/04/10	Sankt-Vith (Eiterbach site 14)
RW057	Belgium- Wallonia	15/04/10	Sankt-Vith (Neidingen)
C16	Luxembourg		Clervaux
Lux001	Luxembourg	02/09	Stolzembourg
Lux002	Luxembourg	23/10/09	Cornelysmillen (Troisvierges)
Lux004	Luxembourg	02/06	Stolzembourg
Lux009	Luxembourg	19/03/10	opposite Gemünd
Lux014	Luxembourg	14/12/09	Ernz Blanche
Lux015	Luxembourg	30/11/09	Erpeldange
Lux016	Luxembourg	4/12/09	Wasserbillig
Lux018	Luxembourg	29/01/10	Moulin de Kalborn
Lux019	Luxembourg	12/02/10	Moulin de Kalborn
Lux021	Luxembourg	24/02/10	Moulin de Kalborn
Am02	Illinois, USA	?	Taush
Am03	Illinois, USA	?	EFF
Am05	Illinois, USA	?	Menard
Am06	Illinois, USA	?	Menard
Am07	Illinois, USA	?	Old Fellow Rd
Am08	Illinois, USA	?	Menard
Am09	Illinois, USA	?	Toles sta
Ger01	Germany	5/12/09	Harspelt
Ger05	Germany	9/12/09	Masthorn
Ger07	Germany	?	Habscheider Mühle
Ger012	Germany	?	Ihrenbrück
Ger013	Germany	9/12/09	Habscheider Mühle
Ger014	Germany	9/12/09	Alfbach
Ger015	Germany	?	Ihrenbrück
Ger016	Germany	15/02/10	Masthorn
Ger23	Germany	3/04/09	Eifel zoo (removed animal)
Ger25	Germany	3/04/09	Eifel zoo (removed animal)
Ger30	Germany	31/08/10	Marmagen Eifel (Nordrhein-Westfalen)
Fin01	Finland	?	?
Fin03	Finland	?	?
Fin04	Finland	?	?

Appendix 2

Number of alleles, observed/expected heterozygosity and Hardy-Weinberg equilibrium (HWE) values, within genetic clusters and sampling locations (countries). GR cluster = all specimens from Western Europe excluding Finland; Am-Fin cluster = all specimens from Finland and USA; Bel = Belgium; Ger = Germany; Lux = Luxemburg; Fin = Finland; USA = United States of America.

	Number of alleles	Observed heterozygosity	Expected heterozygosity	HWE
GR cluster	43	0,31	0,38	/
Am-Fin cluster	60	0,38	0,56	0,0001
Bel	30	0,32	0,37	/
Lux	35	0,31	0,32	/
Ger	37	0,30	0,35	/
Fin	26	0,29	0,29	
USA	53	0,41	0,53	0,072