Published by Associazione Teriologica Italiana
Volume 32 (2): 203–206, 2021
Hystrix, the Italian Journal of Mammalogy

Available online at:
http://www.italian-journal-of-mammalogy.it
doi:10.4404/hystrix-00444-2021

Short Note

Close spatial overlap between the genetic population boundaries of raccoons and the distribution of the raccoon roundworm in Germany

Alain C. Frantz[1,*], Anna Schleimer[1], Julian Wittische[1,2], Mike Heddergott[1]

[1] *Musée National d'Histoire Naturelle, Luxembourg*
[2] *Fondation Faune-Flore, Luxembourg*

Keywords:
emerging infectious disease
invasive species
public health
spatial genetics
wildlife genetics
zoonosis

Article history:
Received: 17 April 2021
Accepted: 09 August 2021

Acknowledgements
The study was funded by an internal grant from the Musée National d'Histoire Naturelle, Luxembourg. JW was funded by the Luxembourg research fund FNR (C20/SR/14748041). We would like to thank all the hunters of the regional district hunting associations and foresters in Germany and Luxembourg for providing us with samples.

Abstract

While raccoons (*Procyon lotor*) cluster into five genetic populations in Germany, the precise spatial extent and degree of overlap of these clusters are unknown. The raccoon roundworm (*Baylisacaris procyonis*), a parasitic nematode that can cause severe disease in humans, is known to occur in central Germany in two of these five populations. Here we confirm a close alignment between the geographic distribution of the roundworm and the spatial extent of the genetic populations of raccoons in which the parasite occurs. We found little evidence that linear landscape features substantially limit gene flow between populations. Given the large amount of genetic admixture at cluster boundaries, the raccoon roundworm is likely to spread to roundworm-free raccoon populations in the future.

The raccoon (*Procyon lotor*) is a North and Central American mesocarnivore that has become invasive in parts of Asia and Europe (Salgado, 2018). Raccoons are especially common in Germany, where their abundance and geographic distribution increased over the past three decades. The animals are likely to colonise most of Germany by mid-century (Fischer et al., 2016). Recent genetic studies have shown that raccoons in Germany (and Luxembourg) descended from at least five different founder events (Heddergott et al., 2020; Fischer et al., 2015). Distinct clusters were inferred to be present in Saxony (eastern Germany), in Brandenburg and adjoining areas (northeastern Germany), around the Harz low mountains (central Germany), in Hessen and adjoining areas (central Germany), as well as in western Germany and Luxembourg (Fig. 1).

The raccoon roundworm (*Baylisacaris procyonis*) was introduced to Europe alongside the raccoon, its definitive host (Heddergott et al., 2020). The eggs of this parasitic nematode are excreted with raccoon droppings and, if ingested, can cause severe disease in a range of vertebrates, including humans (Kazacos, 2001). In Germany, the parasite occurs in the Hessen and Harz raccoon populations, but not elsewhere (Heddergott et al., 2020). More generally, Stope (2019) speculated that the different German raccoon founder populations might be characterised by distinct (zoonotic) parasite communities. From a public health perspective, understanding the precise geographic extent of the different raccoon populations and their degree of overlap is important to delimitate risk areas for specific pathogens.

The sampling in previous population genetic studies was too patchy to infer the precise spatial extent of the genetic clusters of German raccoons (Heddergott et al., 2020; Fischer et al., 2015). We therefore revisited the population genetic structure of raccoons in Germany with numerous additional samples to (1) infer the spatial extent of the genetic populations and their degree of overlap, (2) to identify landscape features that limit functional connectivity between clusters, as well as (3) to compare the geographic distribution of the roundworm to that of the different raccoon populations.

Between 2011 and 2016, we collected 859 tissue samples from road-killed or legally harvested raccoons (Fig. 1), including 365 individuals that had already been analysed by Heddergott et al. (2020) and Fischer et al. (2015). We followed the methodology outlined by Osten-Sacken et al. (2018) to generate 17-microsatellite-based genetic profiles. Working with a subsample of the present dataset, Fischer et al. (2015) found no evidence for systematic deviations from Hardy-Weinberg and linkage equilibria in these 17 loci.

We estimated the most likely number of genetic clusters (K) using STRUCTURE v. 2.3.4 (Pritchard et al., 2000), conducting ten independent runs of K=1-10, with other analysis parameters set as in Osten-Sacken et al. (2018). The most probable number of clusters was chosen based on the ten log-likelihood values inferred for each K and their convergence across runs. After accounting for label switching and confirming the lack of multimodality, the assignment probability (q) of each individual to the detected clusters was averaged across different runs.

We also analysed the data using the Bayesian clustering model implemented in GENELAND v.4.0.8 (Guillot et al., 2005) to incorporate geographic coordinates during inference. We performed ten initial runs to infer the optimal number of K, allowing K to vary between 1-10, followed by a further 100 runs with K fixed to the inferred number of clusters. In each run, we used 10^6 Markov Chain Monte Carlo (MCMC) iterations, a thinning interval of 10^3, a maximum rate of the Poisson process of 862, a maximum number of nuclei in the Poisson-Voronoi tessellation fixed at 2586 and an uncertainty attached to the spatial coordinates of 2 km. The Dirichlet model served as allele fre-

---

[*] Corresponding author
Email address: `alain.frantz@mnhn.lu` (Alain C. Frantz)

doi:10.4404/hystrix-00444-2021
16th December 2021

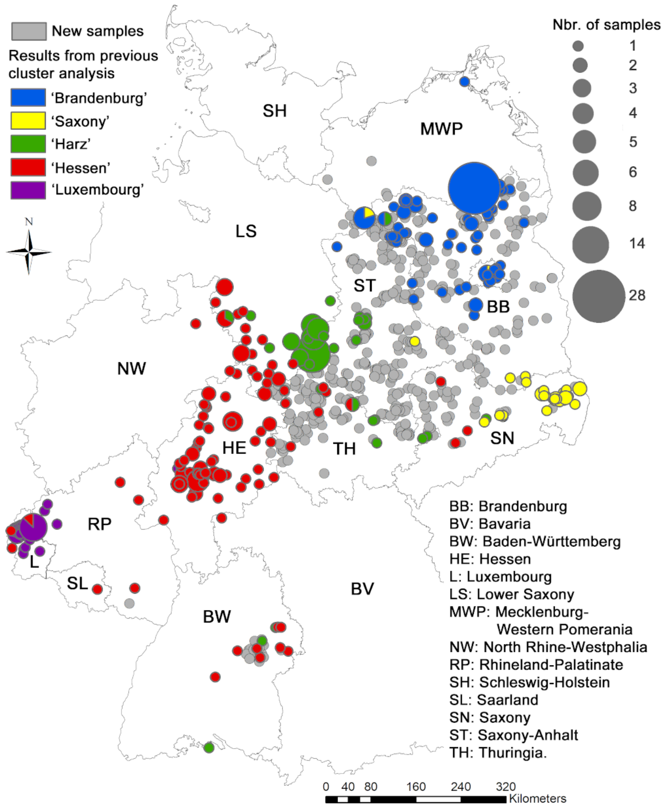

Figure 1 – Geographic origin of the 859 raccoons included in the study. The pie charts represent a previous analysis of genetic structure using a sub-sample of the present dataset (Heddergott et al., 2020), where different colours represent different genetic populations. The size of the pie chart is indicative of the number of samples analysed per locality.

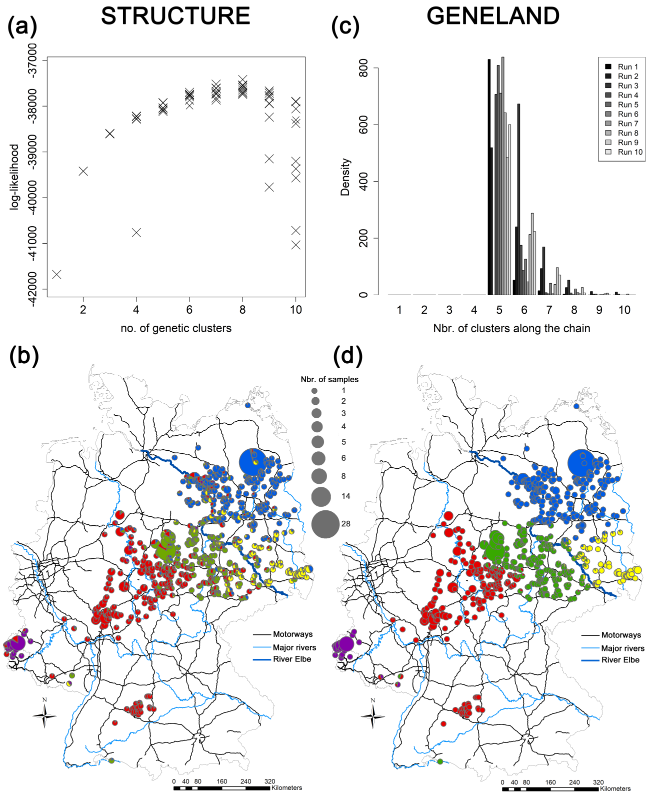

Figure 2 – Analysis of the population genetic structure of raccoons in Germany. STRUCTURE analysis: (a) Plot of the number of STRUCTURE clusters K against their estimated log-likelihood. Crosses represent the results from the ten independent runs performed for each value of K.(b) Geographic distribution of the genetic clusters inferred with the STRUCTURE algorithm for K=5. GENELAND results: (c) Results of the ten initial runs performed to infer the optimal number of genetic clusters K, where K varied between 1-10. (d) Geographic distribution of the five genetic clusters inferred with the GENELAND algorithm. Different colours represent different genetic populations, with the same colour referring to the same genetic cluster. In each map, pie charts represent the average per cluster assignment values for all the individual(s) from a specific location and their size is indicative of the number of samples included.

quency prior. The posterior probabilities of population membership were averaged across the ten runs with the highest log-likelihood values. The spatial configuration of the inferred genetic clusters of raccoons was visually compared to the presence of major rivers and motorways, which may act as barriers to gene flow (Cullingham et al., 2009; Santonastaso et al., 2012).

Each individual was assigned to the STRUCTURE cluster for which it had the highest percentage of membership. We then performed a factorial correspondence analysis (FCA) in GENETIX v.4.05.2 (Belkhir et al., 2004) to visualise the genetic distance between the inferred STRUCTURE clusters. The degree of genetic divergence between STRUCTURE clusters was quantified using F_{ST} (Weir and Cockerham, 1984) in SPAGEDI v.1.5 (Hardy and Vekemans, 2002) and significant difference from zero was tested with 10,000 permutations of individual genotypes between clusters. SPAGEDI v.1.5 was also used to test for the presence of an isolation-by-distance (IBD) pattern by regressing pairwise estimates of Loiselle's kinship coefficient F_{ij} (Loiselle et al., 1995) against the natural logarithm of inter-individual geographic distances. The slope of this regression, which estimates the degree of spatial autocorrelation, was tested for significant difference from zero by 10,000 permutations of the locations of the individuals. We tested for IBD in the complete dataset and within the inferred genetic clusters.

In order to compare the current range of the raccoon roundworm to the spatial extent of the genetic clusters of raccoons, we plotted the roundworm records of Heddergott et al. (2020), obtained from analysing 8,184 raccoons, onto the 10x10k̃m ETRS89-LAEA5210 reference grid of the European Environment Agency.

STRUCTURE did not provide clear support for a specific number of clusters (Fig. 2a). The highest log-likelihood values (K=7, K=8) converged poorly. At K=6, six log-likelihood values converged better, but one of the inferred clusters had assignment probabilities of $q \leq 0.68$ and was probably an artefact. When considering the four runs that converged around the higher log-likelihood value that was detected for K=5, STRUCTURE inferred five clusters that appeared to correspond to the previously identified 'Brandenburg', 'Harz', 'Hessen', 'Luxembourg' and 'Saxony' populations, respectively (compare Fig. 1 to Fig. 2b). Specifically, the Brandenburg cluster was mainly located in north-eastern Germany and the Saxony cluster in eastern Saxony. The Hessen cluster contained animals from central and southern Germany and the Harz cluster extended from central into southern eastern Germany. The fifth cluster was confined to Luxembourg and the surrounding German areas. The boundaries between the STRUCTURE clusters were not clearly defined (especially in the case of the Harz, Hessen and Saxony clusters) as the program inferred a substantial amount of admixture at cluster edges (Fig. 2b).

GENELAND inferred the presence of K=5 genetic clusters in nine of the ten initial runs (with the remaining one inferring K=6; Fig. 2c). The five inferred clusters corresponded essentially to five STRUCTURE clusters (compare Figs. 2b & 2d). Visual inspection suggested that the boundaries of the GENELAND clusters did not correspond to linear landscape features (Fig. 2d), with the possible exception of the boundary between the Harz and the Saxony clusters, which appeared to be associated with the course of a major river, the Elbe (Fig. 2d).

In the FCA (Fig. 3), the Brandenburg, Harz and Hessen clusters were distinct, but the Saxony cluster overlapped somewhat with the Brandenburg and Harz clusters. The Luxembourg cluster overlapped completely with Hessen. Pairwise F_{ST} estimates ranged from F_{ST}=0.034 to F_{ST}=0.141 (Tab. 1). The lowest genetic differentiation was observed between the Harz, Hessen and Saxony clusters ($0.034 \leq F_{ST} \leq 0.048$). The whole dataset was characterised by a significant IBD pattern (slope ± s.e.= -0.0183 ± 0.0030, $p \leqslant 0.001$). However, compared to the overall dataset, the slope of the IBD pattern was less steep within three clusters

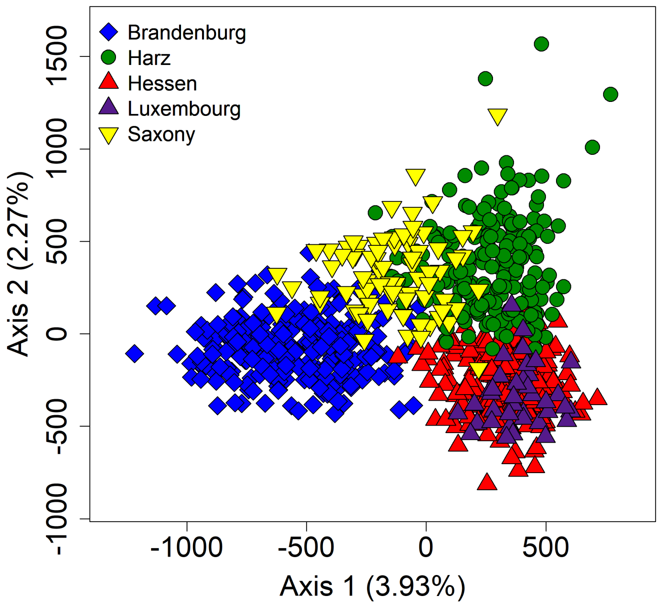

Figure 3 – Factorial correspondence analysis of the microsatellite-based genetic profiles generated for 859 raccoons from Germany. The percentage of the total variation explained by each of the two axes is indicated.

(Brandenburg: slope ± s.e.=-0.0053 ± 0.0011, $p\leqslant 0.001$; Harz: slope ± s.e.=-0.0028 ± 0.0007, $p\leqslant 0.001$; Hessen: slope ± s.e.=-0.0026 ± 0.0007, $p\leqslant 0.001$) and even not significantly different from zero in the case of the Saxony cluster (slope ± s.e.=-0.0015 ± 0.0007, p=0.231).

There generally was a close match between the geographic distribution of *B. procyonis* in Germany and the Hessen and Harz raccoon genetic clusters (Fig. 4). However, in the admixture area between the Harz and Saxony clusters in southern eastern Germany (eastern Thuringia and western Saxony), a larger number of raccoons assigned to the STRUCTURE Harz cluster were located to the east of the *B. procyonis* distribution area. Also, the north-eastern edge of the *B. procyonis* distribution range slightly extended into the Brandenburg cluster in an area of admixture between the Harz and Brandenburg clusters (Fig. 4).

The Bayesian clustering methods inferred the presence of the same five genetic populations as previously identified by Heddergott et al. (2020) and Fischer et al. (2015) and referred to as ‘Brandenburg’, ‘Harz’, ‘Hessen’, ‘Saxony’ and ‘Luxembourg’, respectively. The results generally support the conclusion of Fischer et al. (2015) that the clusters arose from independent introduction events. While the whole dataset was characterised by a significant IBD pattern, the pattern within clusters was much weaker. This result suggested that the IBD pattern observed across the entire study area resulted from the presence of genetic discontinuities between clusters rather than from a true IBD gradient. Both of these effects (and combinations thereof) can lead to a significant regression between the geographic and the genetic distance matrices (Guillot et al., 2009). Also, there was generally little overlap between the different FCA clusters and all F_{ST} estimates were high ($F_{\mathrm{ST}}\geq 0.034$) compared to estimates reported from rural populations in the species’ native range (F_{ST}=0.0019-0.0220; Côté et al., 2012; Cullingham et al., 2009; Dharmarajan et al., 2009; Root et al., 2009). While Santonastaso et al. (2012) reported higher F_{ST} estimates (0.057-0.078) for raccoon populations in an urban area, these authors speculate that these values resulted from the presence of migrant or translocated raccoons.

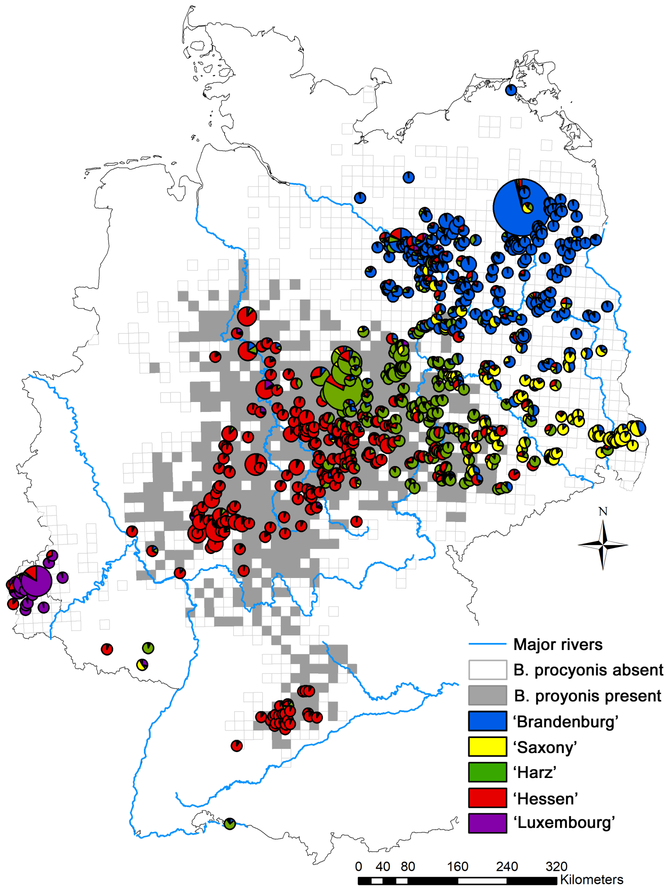

Figure 4 – Comparison of the geographic distribution of the raccoon roundworm with the spatial extent of the STRUCTURE clusters inferred for K=5. The roundworm data are based on the analysis of 8,184 raccoons (Heddergott et al., 2020) and presence/absence of the parasite are plotted using the 10x10 km ETRS89-LAEA5210 EEA reference grid.

The lowest F_{ST} estimates were obtained for comparisons involving the Harz, Hessen and Saxony clusters. STRUCTURE inferred a substantial amount of admixture at the joint boundaries of the three clusters. Moreover, raccoons in eastern Saxony have mitochondrial control region haplotypes not observed elsewhere in Germany (Frantz et al., 2013) and historical records support an independent introduction of raccoons in the Harz Mountains (Stubbe, 1975). Additionally, the raccoon roundworms in Hessen and around the Harz also form two distinct genetic populations (Osten-Sacken et al., 2018). Considering all this evidence, the Harz, Hessen and Saxony clusters probably resulted from independent introduction events, but were genetically less differentiated because of admixture with adjoining populations.

While the IBD pattern within clusters was relatively weak, it may explain the artificial clusters observed with program STRUCTURE at K>5. STRUCTURE infers genetic populations by minimising deviations from Hardy-Weinberg and linkage equilibria within a dataset.

Table 1 – Estimates of pairwise genetic differentiation between the STRUCTURE-inferred cluster. FST estimates according to Weir and Cockerham (1984) below diagonal, significance values above diagonal.

Cluster	Brandenburg	Harz	Hessen	Luxembourg	Saxony
Brandenburg		<0.001	<0.001	<0.001	<0.001
Harz	0.088		<0.001	<0.001	<0.001
Hessen	0.090	0.034		<0.001	<0.001
Luxembourg	0.141	0.077	0.090		<0.001
Saxony	0.058	0.048	0.041	0.119	

The algorithm can thus identify artificial clusters when faced with deviations from random mating that do not result from genetic discontinuities, as is the chase in a dataset characterised by IBD (Frantz et al., 2009). In order to provide biologically meaningful information about a species, IBD analyses should be limited to individuals of reproductive age, as the inclusion of pre-dispersal juveniles biases the results towards greater correlation between spatial and genetic distances (Coltman et al., 2003). Since we did not have information on the age categories of the raccoons, it was not possible to make robust inferences about the species' genetic neighbourhood size and its potential influence on disease transmission (e.g., Storfer et al., 2017).

Visual inspection of the association of genetic boundaries with linear landscape features provided very little evidence for landscape features acting as a substantial barrier for raccoon dispersal. There appeared to be a close match between cluster boundaries and the river Elbe in southern eastern Germany, but animals assigned to the same cluster were found on both side of the river Elbe further to the north. The river Elbe may thus represent a barrier, but its permeability may change along its course. Genetic studies from the raccoons' native range in North American have shown that large rivers may act as movement barriers, but the strength of the barrier effect depends on a river's physical characteristics (Côté et al., 2012; Cullingham et al., 2009). Other studies from North America did not find evidence of the genetic structure at a local scale in rural areas being affect by landscape barriers (Dharmarajan et al., 2009; Root et al., 2009, see also Santonastaso et al., 2012). Explicitly modelling the landscape resistance using a genetics-based approach (e.g. Kimmig et al., 2020) would help to empirically infer the permeability of the river Elbe to raccoon movement and perhaps identify other landscape features that affect gene flow in raccoons.

The close match between the geographic distribution of *B. procyonis* and the Harz and Hessen raccoon clusters supports the hypothesis that the presence of the parasite in an introduced raccoon population is dependent on the initial infection status of the founder individuals (Heddergott et al., 2020). However, the apparent lack of effective barriers (with the possible exception of the river Elbe) and the large amount of admixture in contact areas between populations suggest a likely spread of the nematode into roundworm-free populations in the near future. Indeed, our results suggested that dispersing raccoons have already spread *B. procyonis* into areas that were previously roundworm-free. It is not clear at present whether the parasite has established itself in these areas or whether these findings were incidental and limited to immigrants. In the longer term, it appears likely that the parasite fauna of the different raccoon populations will homogenise. A continuous monitoring of *B. procyonis*, along with public health awareness efforts, ought to be considered in previously roundworm-free areas, especially in areas of contact between the different genetic populations.

References

Belkhir K., Borsa P., Chikhi L., Raufaste N., Bonhomme F., 2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, France.

Coltman D.W., Pilkington J.G., Pemberton J.M., 2003. Fine-scale genetic structure in a free-living ungulate population. Mol. Ecol. 12(3): 733–742

Côté H., Garant D., Robert K., Mainguy M., Pelletier F., 2012. Genetic structure and rabies spread potential in raccoons: the role of landscape barriers and sex-biased dispersal. Evol. Appl. 5(4):393-404

Cullingham C. I., Kyle C.J., Pond B.A., Rees E.E., White B.N., 2009. Differential permeability of rivers to raccoon gene flow corresponds to rabies incidence in Ontario, Canada. Mol. Ecol. 18(1): 43–53.

Dharmarajan G., Beasley J.C., Fike J.A., Rhodes O.E. Jr., 2009. Population genetic structure of raccoons (*Procyon lotor*) inhabiting a highly fragmented landscape. Can. J. Zool. 87(9): 814–824.

Fischer M.L., Hochkirch A., Heddergott M., Schulze C., Anheyer-Behmenburg H.E., Lang J., Michler F.U., Hohmann U., Ansorge H., Hoffmann L., Klein R., Frantz A.C., 2015. Historical invasion records can be misleading: genetic evidence for multiple introductions of invasive raccoons (*Procyon lotor*) in Germany. PLoS One 10(5): e0125441.

Fischer M.L., Sullivan M.J.P., Greiser G., Guerrero-Casado J., Heddergott M., Hohmann U., Keuling O., Lang J., Martin I., Michler F.U., Winter A., Klein R., 2016. Assessing and predicting the spread of non-native raccoons in Germany using hunting bag data and dispersal weighted models. Biol. Invasions 18(1): 57–71.

Frantz A.C., Cellina S., Krier A., Schley L., Burke T., 2009. Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? J. Appl. Ecol. 46 (2): 493–505.

Frantz A.C., Heddergott M., Lang J., Schulze C., Ansorge H., Runge M., Braune S., Michler F.U., Wittstatt U., Hoffmann L., Hohmann U., Michler B.A., Van Den Berge K., Horsburgh G.J., 2013. Limited mitochondrial DNA diversity is indicative of a small number of founders of the German raccoon (*Procyon lotor*) population. Eur. J. Wildl. Res. 59(5): 665–674.

Guillot G., Estoup A., Mortier F., Cosson J., 2005. A spatial statistical model for landscape genetics. Genetics, 170(3): 1261–1280.

Guillot G., Leblois R., Coulon A., Frantz A.C., 2009, Statistical methods in spatial genetics. Mol. Ecol. 18(23):4734–4756.

Hardy O.J., Vekemans X., 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes 2(4): 618–620.

Heddergott M., Steinbach P., Schwarz S., Anheyer-Behmenburg H.E., Sutor A., Schliephake A., Jeschke D., Striese M., Müller F., Meyer-Kayser E., Stubbe M., Osten-Sacken N., Krüger S., Gaede W., Runge M., Hoffmann L., Ansorge H., Conraths F.J., Frantz A.C., 2020. Geographic distribution of raccoon roundworm, *Baylisascaris procyonis*, Germany and Luxembourg. Emerg. Infect. Dis. 26(4): 821.

Kazacos K.R., 2001. *Baylisascaris procyonis* and related species. In: Samuels W.M., Pybus M.J., Kocans A.A. (Eds.) Parasitic diseases of wild mammals. 2nd ed. Iowa State University Press, Ames, IA. 301–341.

Kimmig S.E., Beninde J., Brandt M., Schleimer A., Kramer-Schadt S., Hofer H., Börner K., Schulze C., Wittstatt U., Heddergott M., Halczok T., Staubach C., Frantz A.C., 2020. Beyond the landscape: Resistance modelling infers physical and behavioural gene flow barriers to a mobile carnivore across a metropolitan area. Mol. Ecol. 29(3): 466–484.

Loiselle B.A., Sork V.L., Nason J., Graham C., 1995. Spatial genetic structure of a tropical understorey shrub, *Psychotria officinakus* (Rubiacae). Am. J. Bot. 82(11): 1420–1425.

Osten-Sacken N., Heddergott M., Schleimer A., Anheyer-Behmenburg H.E., Runge M., Horsburgh G.J., Camp L., Nadler S.A., Frantz A.C., 2018. Similar yet different: co-analysis of the genetic diversity and structure of an invasive nematode parasite and its invasive mammalian host. Int. J. Parasitol. 48(3-4): 233–243.

Pritchard J.K., Stephens M., Donnelly P., 2000. Inference of population structure using multilocus genotype data. Genetics 155(2): 945–959.

Root J.J., Puskas R.B., Fischer J.W., Swope C.B., Neubaum M.A., Reeder S.A., Piaggio A.J., 2009. Landscape genetics of raccoons (*Procyon lotor*) associated with ridges and valleys of Pennsylvania: implications for oral rabies vaccination programs. Vector Borne Zoonotic Dis. 9(6):583–588.

Salgado I., 2018. Is the raccoon (*Procyon lotor*) out of control in Europe? Biodivers. Conserv. 27(9): 2243–2256.

Santonastaso T.T., Dubach J., Hauver S.A., Graser W.H. III, Gehrt S.D., 2012. Microsatellite analysis of raccoon (*Procyon lotor*) population structure across an extensive metropolitan landscape. J. Mammal. 93(2): 447–455.

Stope M., 2019. Wild raccoons in Germany as a reservoir for zoonotic agents. Eur. J. Wildl. Res. 65(6): 94.

Storfer A., Epstein B., Jones M., Micheletti S., Spear S. F., Lachish S., Fox S., 2017. Landscape genetics of the Tasmanian devil: implications for spread of an infectious cancer. Conserv. Genet. 18(6):1287–1297.

Stubbe M., 1975, Der Waschbär *Procyon lotor* (L., 1758) in der DDR. Hercynia N. F. 12(1): 80–91.

Weir B.S., Cockerham C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38(6): 1358–1370.

Associate Editor: M. Musiani