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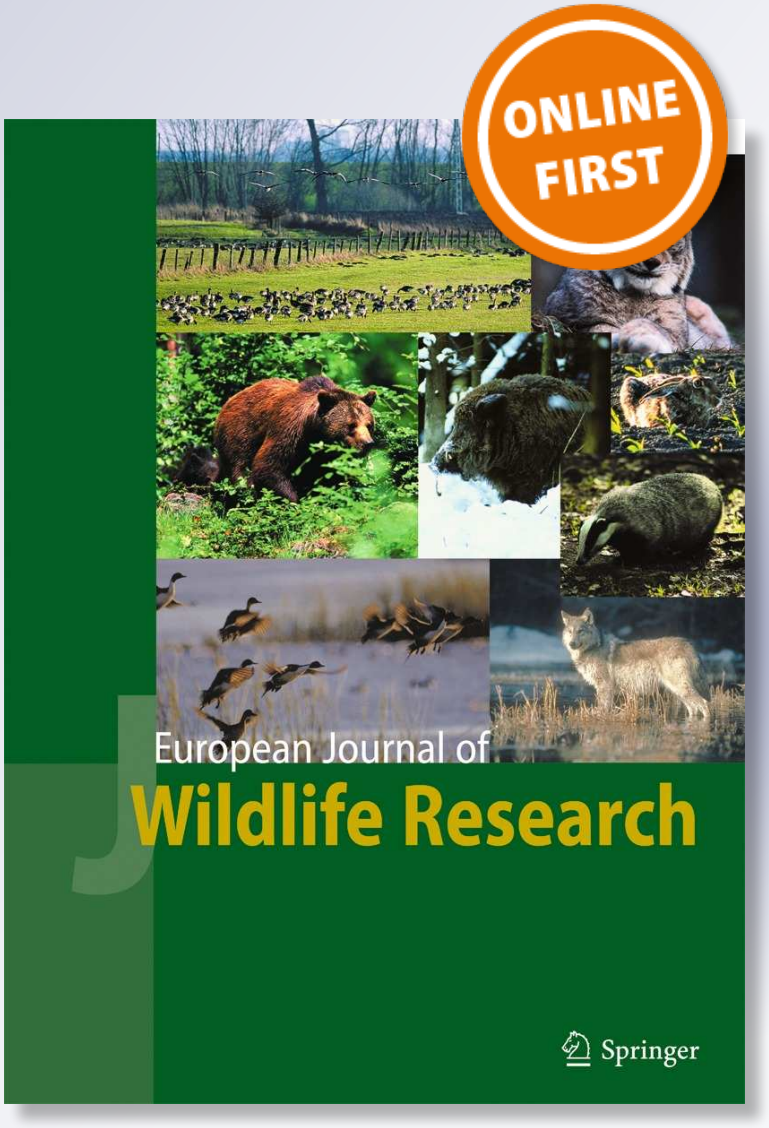

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ORIGINAL PAPER

Roe deer population structure in a highly fragmented landscape

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Abstract Northern Belgium (Flanders) is one of the most densely populated and urbanized regions in Europe. Many species are therefore likely to suffer from anthropogenic pressure and habitat destruction and fragmentation. Although many large mammals are recolonizing in parts of Europe, including Belgium, due to adaptation, a relaxation of persecution and habitat restoration, we have little actual data concerning the effects of landscape features on their population structure. We analysed the genetic structure of discrete roe deer (*Capreolus capreolus*) populations in the Eastern part of Flanders, with special emphasis on the impact of habitat fragmentation and anthropogenic barriers. The sampled populations were clearly genetically differentiated. Genetic structure could be explained by purely distance-based landscape modelling, but a simpler model focusing solely on barrier effects of large transportation infrastructure explained nearly as much genetic variance. In contrast, analyses based on least-cost landscape modelling failed to yield a significant effect. Overall, the results suggest considerable landscape-level effects of transportation infrastructure.

Keywords *Capreolus capreolus* · Fragmentation · Genetic drift · Redundancy analysis · Landscape genetics

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Introduction

The Western European landscape has changed drastically during the last decades resulting in both landscape homogenization and fragmentation. The increasing road density and intensity of its use, and the building of new railroads, have led to an increase of barriers in the landscape (Antrop 2004). Several mammals are hampered in their movements by urban areas, roads and unattractive land (Jongman 2002; Frantz et al. 2012), while habitat destruction due to anthropogenic pressures results in both habitat loss and habitat fragmentation (Bright 1993; Fahrig 2003). Though habitat fragmentation per se can have both positive and negative effects on animal abundance and surviving, the net outcome is usually negative (Andren 1994, Jaeger et al. 2011). In Flanders, the northern part of Belgium, anthropogenic pressures have resulted in one of the highest habitat fragmentation levels of Europe (Jaeger et al. 2011). Flanders is one of the most densely populated regions worldwide (462 inhabitants km^{-2}) with scattered urban areas (25 % of the surface) alternating with agricultural areas (62 % of the surface). Forested areas represent around 10 % of the total surface. With a road density of around 5 km km^2 and many railways, linear fragmentation of the landscape is extremely significant (Tromcé et al. 2002). Furthermore, the landscape is intersected by a series of canals with steep borders, making them important barriers even for larger mammals capable of swimming. A landscape fragmentation study in Europe (Jaeger et al. 2011) showed that Northern Belgium, together with the adjacent parts of northwestern France (French Flanders) and western Germany (North Rhine Westphalia) can be considered as a single uninterrupted stretch of extremely fragmented landscape. For many wildlife species in this area, this likely result in small isolated populations where dispersal, necessary for gene flow and (re)colonisation after local extinction, is strongly impeded (Hanski 1998).

While the impact of land use and landscape features on animal dispersal and gene flow is widely accepted, it is only with the emergence of studies within the field of landscape genetics that a direct link between landscape features and genetic structure could be quantified and proven (Manel et al. 2003; Holderegger and Wagner 2008). Several studies have shown that anthropogenic barriers and unsuitable landscape features impede gene flow (Fahrig 2003; Epps et al. 2005; Balkenhol and Waits 2009; Frantz et al. 2012) even for medium-sized ungulate species such as roe deer which are expected to be very mobile (Coulon et al. 2006; Kuehn et al. 2007). In spite of the heavy anthropogenic pressure, the high mobility and dispersal capacity of several medium-sized mammals are exemplified by the recent surge of recolonization of previously abandoned regions in Western Europe. Wolves (*Canis lupus*), Eurasian lynxes (*Lynx lynx*), Eurasian otters (*Lutra lutra*) and more common species such as wild boars (*Sus scrofa*) and beavers (*Castor fiber*) have re-expanded their ranges in the last decades, some of them even into Flanders (Deinet et al. 2013).

Roe deer is currently the most abundant native free-living ungulate species in Flanders, although this is a recent phenomenon. Roe deer densities and distribution strongly increased after the Second World War. Its distribution in the 1960s was still limited in the northern part of the provinces of Limburg and Antwerp while some relict populations were found in the central part of the country around Brussels and Leuven (Casaer and Licoppe 2010). Most probably, roe deer never totally disappeared from most of these regions. In the 1980s, the species started to recover from its earlier decline, with gradual recolonization of previously abandoned areas (Casaer 2003). Currently, it has a broader distribution range than ever registered before, with local population densities exceeding 30 animals km^{-2} of forested area (Casaer and Licoppe 2010). The expansion can be mainly explained by the lack of predators, changes in the hunting regulation, abundant food availability (year round access to arable land) and the high adaptation capacity of roe deer to new habitat conditions. Undocumented translocations and introduction of animals may also have contributed to the current distribution. Roe deer primarily inhabits woodland areas although it occupies also more open landscapes with woody structures such as hedgerows (Morellet et al. 2011). In fragmented landscapes, roe deer stick to wooded patches and show high site fidelity (Hewison et al. 2001; Cargnelutti et al. 2002). Animals live solitary or in small family groups and exhibit a low level of polygyny. Both sub adult males and females disperse in spring or early summer of the year following birth or in the subsequent spring (Wahlström and Liberg 1995; Linnell et al. 1998). Typical dispersal distances are thought to be low and are mostly restricted to a few kilometres (Linnell et al. 1998), although little is known about dispersal in highly fragmented landscapes. Such a pattern of small-scale dispersal should lead to increasing genetic differentiation with geographic distance (isolation-by-distance). However, in highly fragmented landscapes, it was found that for roe deer genetic distances correlate with urbanization rather than with geographic distance (Wang and Schreiber 2001). The relatively rapid recolonization of formerly abandoned regions nevertheless indicates that the potential to disperse is high, but gene flow dynamics among established populations are potentially low due to behavioural and life history constraints (e.g., Vangestel et al. 2011).

Here, we attempt to identify the important determinants and causes of genetic structuring of roe deer populations across the study region. First, we describe the population genetic structure of roe deer populations in Flanders. We then test which landscape description fits the genetic data best: purely distance-based spatial modelling, the position of populations relative to major transportation infrastructure, or a least-cost analysis that integrates the effect of green landscape features.

Methods

Study area and sampling

The study area is situated in the eastern part of Flanders (Fig. 1) and covers approximately 14,000 km^2 of which 20 % is forested. The study area is intersected by several highways, railways and canals and contains many cities and urbanized regions (Fig. 1). Samples were obtained from nine Game Management Units (GMUs) in the Flemish region (Fig. 1 and Online Resource 1), ranging in size between 19.5 and 99.8 km^2 with a mean of 55.9 km^2. One GMU (Meerdaal) is intersected by a highway (see Online Resource 1), but only animals from the south of the highway were used in this study. As a reference for a stable and large population, samples were included from the region of Elsenborn, located in the Walloon region, about 70 km away from the nearest Flemish sample location. Muscle tissue samples from legally shot animals were collected between 2005 and 2007, and were preserved in 95 % ethanol. More detailed information on sex and age of the samples is given in Online Resource 2.

Genetic analysis

DNA extraction was performed with the DNeasy Tissue Kit (Qiagen) according to the manufacturer's protocol. Ten microsatellite loci (BM757, OarFCB304, BMC1009, HUJ1177, NVHRT48, CSSM41, CSSM43, IDVGA29, CSSM39 and BM1706) were amplified in a single multiplex reaction as described by Galan et al. (2003). PCR amplifications were analysed by capillary electrophoresis on a SCE 9610 genetic

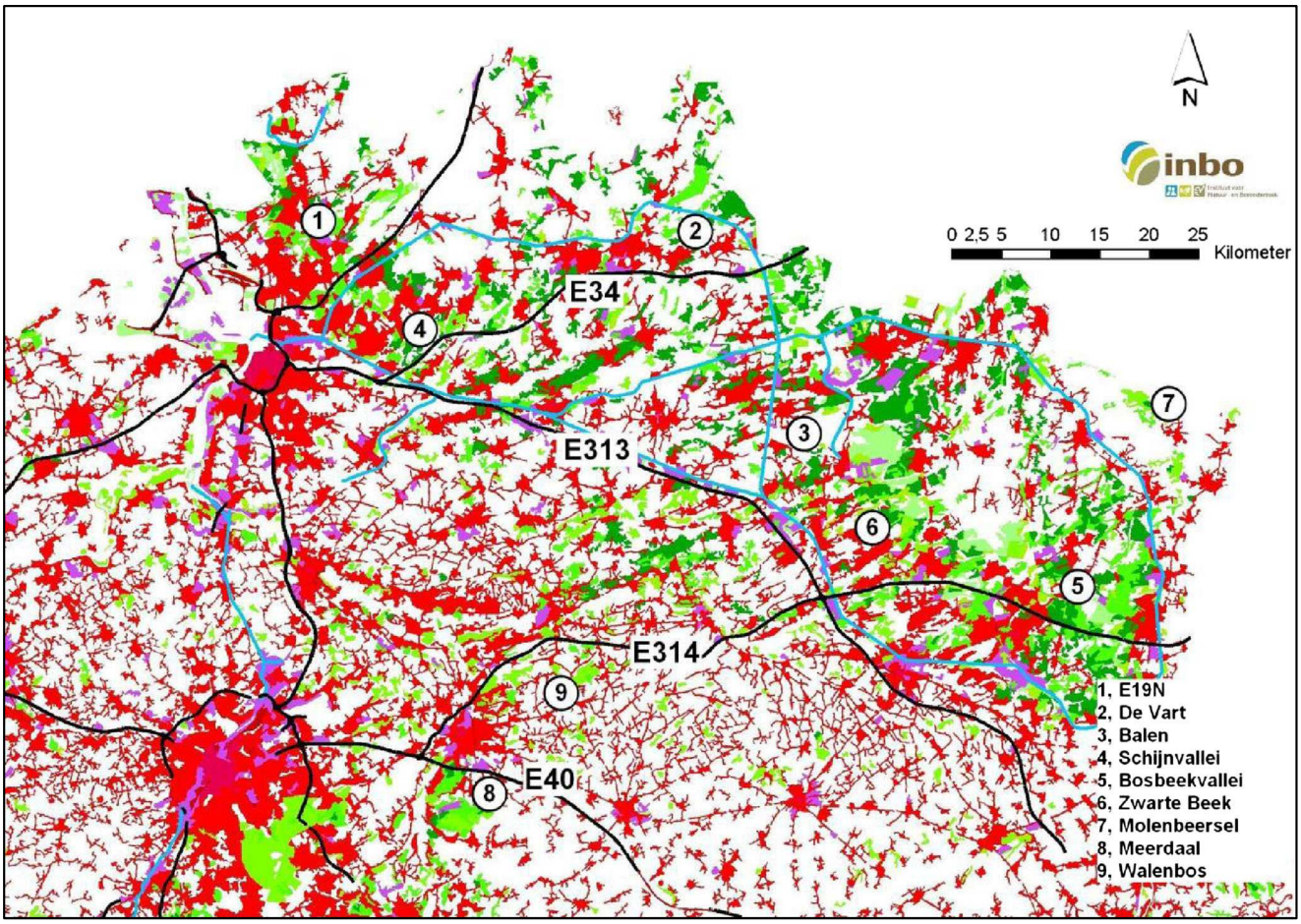

Fig. 1 Sampling area in Eastern Flanders. Urbanized regions are coloured in *red*, industrial zones in *purple*, highways in *black* and canals in *blue*. The names of the highways mentioned in the text are indicated. Forests and other suitable habitats (marshland, heathland,…) are shown in different shades of green

analyzer (Spectrumedix) using the Genospectrum 3.0.0 software.

Data analysis

Genetic diversity and population structure

FSTAT v.2.9.3 (Goudet 2001) was used to infer the number of alleles (NA) and allelic richness (AR) per locus and population as well as to calculate *Fis* and *Fst* values (900 permutations). As alternative for measuring differentiation, D_{est}-values (Jost 2008) were calculated using the programme SMOGD (Crawford 2010) with 999 bootstraps. Observed (H_o) and expected (H_e) heterozygosity were determined according to Nei (1978) using GENETIX v.4.03 (Belkhir 2004). As test for deviation from Hardy-Weinberg equilibrium (HWE), significance of heterozygote deficiency was calculated using GENEPOP v.4.0.10 (Rousset 2008) with 10,000 dememorizations, 300 batches and 10,000 iterations per batch. To visualize potential population structure, a principal coordinate analysis (PCoA) was performed in GENALEX v.6.41 (Peakall and Smouse 2006). The Bayesian assignment approach implemented in STRUCTURE 2.3.2 (Pritchard et al. 2000; Falush et al. 2003) was used to infer the number of genetic clusters in the total sample. For each of K=1 to 11, a model assuming admixture and correlated allele frequencies was run five times with a burn-in period of 100,000 followed by 400,000 MCMC iterations, with prior spatial information provided except for the first run. The genetic structure was inferred based on the mean lnP(D) and by calculating ΔK (Evanno et al. 2005) using STRUCTURE HARVESTER (Earl and von Holdt 2011). GENECLASS2 (Piry et al. 2004) was used to assign individuals to predefined populations (self-assignment) following the partial Bayesian approach of Rannala and Mountain (1997). The Monte Carlo method of Paetkau et al. (2004) was used for exclusion analysis applying default settings.

Spatial genetic structure

We used redundancy analysis (RDA), a constrained ordination method (see Legendre and Legendre 2012 for a comprehensive overview), to analyze the genetic structure of populations (dependent variables) relative to three explanatory variables: (1)

the spatial position in the landscape, (2) major transportation infrastructure in the region and (3) three measures of least-cost distances. The first model adopts distance as the primary driver of genetic structure (isolation by distance), the second assumes that highways and canals are the primary drivers of genetic structure (isolation by barriers) and the third that both distance and the permeability of different landscape features matter.

Least cost path analysis was performed in ARCGIS v.10 (ESRI) using the Spatial Analyst tools. A cost raster was first created using a land use map (Gobin et al. 2009) with cells of 15×15 m and assigning costs to each cell based on the presence of predefined barriers and landscape features (Table 1). We preferred to work with such small cell sizes as it is easier to deal with linear barrier elements such as high ways and canals, what was very important in our study given the fact that the study area contains many barriers. To weight the effect of geographical distances to a varying degree when calculating the least cost path, three least cost matrices (LCM) were generated with cost values of 1 to 10 (1, no resistance; 5, intermediate resistance and 10, absolute barrier; LCM10), 1 to 100 (1, no resistance; 50, intermediate resistance and 100, absolute barrier; LCM100) and 1 to 1000 (1, no resistance; 500, intermediate resistance and 1000, absolute barrier; LCM1000). The cost value of each landscape feature was determined based on the estimated permeability or barrier effect for roe deer (Table 1). Next, a cost distance raster was created for each of the sampling areas (polygon) equalling per raster cell the cumulative cost of reaching the centre of the polygon starting from that cell. As we had no individual coordinates of the shot animals, we used the polygon centroids of each sampling area as geographical coordinates. This might have caused some imprecision, but since most distances between the different game management units are far much bigger than the distances between individual animals within a unit, we didn't expect too much bias. Finally, least cost paths between each pair of sampling areas were calculated, resulting in the final least cost path matrices.

Table 1 Predefined barriers and landscape features and assigned cost values

Barrier/landscape feature	Cost value
Residential and commercial area	10, 100, 1000
Industrial area	10, 100, 1000
Highway and railway	10, 100, 1000
Main river and canal	5, 50, 500
Grassland and arable land	5, 50, 500
Park and recreation zone	5, 50, 500
Military zone	5, 50, 500
Forest	1
Marshland	1
Heathland	1

For the RDA, we first calculated Nei genetic distances (Nei 1972) among populations in GENALEX v.6.4.1 and performed principal coordinate analyses (PCoA) on these distances using PrCoord 1.0 in the Canoco 4.5 software package (ter Braak and Šmilauer 2002). All resulting PCoA axes with positive eigenvalues were used as the dependent variables in the RDA. The purely spatial effect was investigated using distance-based Moran Eigenvector Maps (db-MEM) (Dray et al. 2006; Legendre and Legendre 2012). Also for the least cost models, MEMs were calculated based on pairwise least-cost distances between all sampled locations. Since we had no detailed landscape information for the Elsenborn region, this population was omitted from these analyses. For comparative purposes, the other RDA analyses were performed with and without this population. For the road infrastructure models, the position of sampled locations relative to the linear infrastructure was coded as a binary (dummy) explanatory variable. A population is thus located on one side of the infrastructure (having value=1) or on the other side (value=0). Having only sampled ten populations, the number of highways we can test is rather limited, as we need a minimum of two, but preferably more, populations on each side of the investigated infrastructure. We chose the E34 highway and the E313 plus E314 highways (with the E313 coinciding completely with the large and broad Albert canal), as they represent the most obvious testable linear barriers (Fig. 1). We chose not to include Mantel tests, as these are generally weak at detecting spatial patterns (Legendre et al. 2005; Gilbert and Bennett 2010; Legendre and Fortin 2010).

We used the double stop criterion of Blanchet et al. (2008) for RDA model selection, which consists of testing the significance of the global model (including all explanatory variables), and only continuing to forward selection of variables if this model is significant. Significance testing was done with 999 permutations. For the global RDA model, we report adjusted regression coefficients (R^2_{adj}), thereby correcting for model overfitting (Peres-Neto et al. 2006). All RDA analyses were performed using the rda and varpart functions of the vegan package (Oksanen et al. 2011). In addition, the forward.sel function of the packfor package (Dray et al. 2006) was used. MEM spatial eigenfunctions were computed using the create.MEM.model function in the PCNM package (Legendre et al. 2010). All analyses were done in R v.2.15.1 (www.r-project.org).

Results

Genetic diversity and population structure

Altogether, 536 individuals, divided over the ten sampling regions, were genotyped. In total, 81 alleles were scored with the number of alleles per locus ranging from 3 (IDVGA2) to

Table 2 Basic genetic parameters calculated for the different populations

Pop	Abbr.	N	NA	AR	PA	F_{is}	H_e	H_o
De Vart	DV	55	4.9	6.0	0	−0.005	0.604	0.607
Meerdaal	MD	55	5.5	6.97	1	0.074	0.595	0.551**
Molenbeersel	MB	47	5.0	6.50	0	0.055	0.597	0.564**
Balen	B	43	5.4	7.0	0	−0.008	0.630	0.635
Walenbos	WB	43	4.8	8.0	1	0.084	0.626	0.574*
Elsenborn	EB	58	6.5	9.13	6	0.005	0.659	0.656
Zwarte Beek	ZB	52	5.5	6.74	1	0.033	0.610	0.590
E19N	EN	70	5.0	5.80	0	0.031	0.655	0.635
Schijnvallei	SV	59	5.2	7.22	0	0.077	0.587	0.542**

N sample size, *NA* mean number of alleles per locus, *AR* allelic richness, *PA* private alleles, *Fis* inbreeding coefficient, H_e expected and H_o observed heterozygosity with indication of a significant deviation from Hardy-Weinberg equilibrium at *$p<0.05$ or **$p<0.001$

17 (OarFCB304). Table 2 shows a summary of the main basic genetic parameters per population. The overall level of genetic diversity, measured as mean number of alleles per locus and allelic richness, was slightly higher in the population of Elsenborn than in the Flemish populations. However, except for Molenbeersel (Wilcoxon test, $0.02<p<0.05$), the differences in allelic richness were not significant ($p>0.05$). Also, heterozygosity (H_e and H_o) levels did not significantly differ ($p>0.05$) between populations, except for Molenbeersel and Elsenborn ($0.02<p<0.05$). All populations except Bosbeekvallei, De Vart and Balen displayed a deficit of heterozygotes ($F_{is}>0$), and four populations (Schijnvallei, Meerdaal, Molenbeersel and Walenbos) showed a significant deviation from HWE at several of the loci. The overall F_{st} on the whole data set was 0.075, while pairwise F_{st}-values (Table 3) ranged from 0.031 (E19N with Balen) to 0.14 (Schijnvallei and De Vart with Elsenborn). All F_{st} values were significant at the $p<0.001$ level. From the 45 pairwise comparisons, 16 (36 %) lay between 0.08 and 0.14 indicating moderate differentiation. Pairwise D_{est}-values were largely comparable with the F_{st} values (Table 3) but here, 56 % of the pairwise values were higher than 0.08.

A principal coordinate analysis at the level of the individuals did not show a clear structure (Online Resource 3). At the population level, a pattern of gradual transition from the south-eastern, relict populations (Elsenborn, Molenbeersel and Meerdaal) towards the north-western, more recent ones (E19N, Schijnvallei and Balen), can be distinguished (Fig. 2), with the first principal coordinate largely separating both groups of populations. The same pattern is also reflected in the pairwise F_{st}-values between these populations (Table 3). The mean F_{st}-values amongst the relict populations are all lower than the values of relict populations compared to the younger ones. This pattern is also further supported by Bayesian analysis using STRUCTURE. The most likely partitioning of the genetic diversity based on delta K was obtained with K=2, while a weaker structure was obtained with K=4 (Table 4 and Online Resource 1 and 4). For K=2, the majority of the individuals of Elsenborn, Meerdaal, Bosbeekvallei and Molenbeersel belong to one cluster, while De Vart, Balen, Walenbos, E19N and Schijnvallei form a second cluster. For K=4, more than 95 % of the deer from Elsenborn belong to one distinct cluster. A second cluster comprises most individuals from Meerdaal (89 %) together with around one third of the animals from the nearby

Table 3 Pairwise F_{st} (below diagonal) and D_{est} (above diagonal) values between populations

	BBV	DV	MD	MB	B	WB	EB	ZB	EN	SV
Bosbeekvallei	–	0.106	0.065	0.063	0.055	0.080	0.090	0.041	0.104	0.111
De Vart	0.110	–	0.161	0.113	0.027	0.050	0.218	0.059	0.033	0.051
Meerdaal	0.068	0.107	–	0.057	0.120	0.083	0.097	0.077	0.128	0.105
Molenbeersel	0.056	0.097	0.059	–	0.054	0.119	0.084	0.044	0.098	0.098
Balen	0.061	0.028	0.085	0.060	–	0.065	0.163	0.035	0.039	0.077
Walenbos	0.083	0.075	0.072	0.078	0.060	–	0.140	0.116	0.063	0.082
Elsenborn	0.073	0.139	0.076	0.057	0.100	0.097	–	0.091	0.175	0.211
Zwarte Beek	0.059	0.061	0.060	0.046	0.037	0.095	0.074	–	0.069	0.091
E19N	0.070	0.040	0.089	0.067	0.031	0.061	0.102	0.056	–	0.042
Schijnvallei	0.088	0.044	0.084	0.099	0.061	0.072	0.140	0.082	0.043	–

Abbreviations are as in Table 2

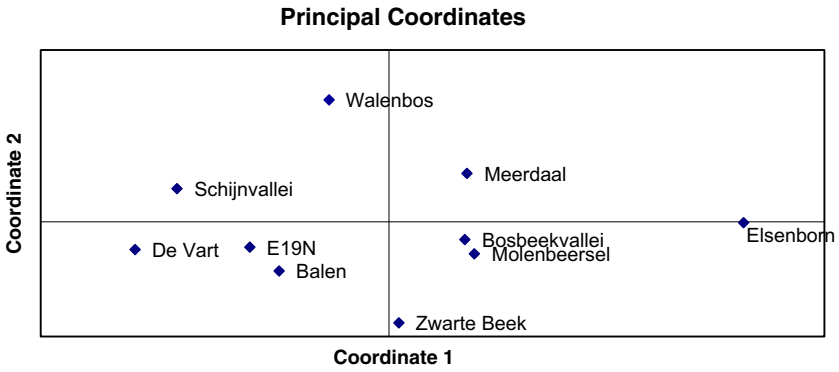

Fig. 2 Principal coordinate analysis of the populations. The first and second coordinate account respectively for 60.15 and 16.16 % of the total variation

Walenbos population. The other populations are divided over two additional clusters largely reflecting the geographic origins with all western populations in one cluster and the more eastern populations in another cluster. Only Balen and Walenbos have a substantial number of individuals in two clusters. According to the assignment probabilities computed in GENECLASS, 70 % of all individuals could be assigned to a specific population with a probability >0.7. Of these animals, between 70 and 98 % were correctly assigned to the predefined population (Table 5) except for Balen (59 %). When considering an assignment probability of more than 90 %, the percentage of correctly assigned individuals dropped below 20 % for three of the populations (De Vart, Balen and Zwarte Beek) and only stayed above 70 % for Meerdaal (73 %) and Elsenborn (83 %) (data not shown). In total, ten animals were excluded from their population of origin, two in Meerdaal and Molenbeersel and one each in the other populations except for Balen en Walenbos where there were no exclusions. Three individuals (one from Molenbeersel (exclusion threshold 0.001) and the ones from Elsenborn (exclusion threshold 0.008) and Schijnvallei (exclusion threshold 0.004)) were excluded from all populations.

Spatial population structure

The redundancy analysis using purely spatial distance-based Moran eigenvector maps, and including Elsenborn, yielded two significant db-MEM-variables in the global model which together explained 18.2 % of the genetic data (p=0.023) (Table 6). Both variables were retained after forward selection. When Elsenborn was excluded, four db-MEM-variables were modelled (R^2_{adj}=0.302, p=0.011). Of these four variables, the first two were sufficient to significantly (p=0.001) explain the adjusted variance. A separate redundancy analysis considering only the highways E34 and E313/E314 (plus Albert canal) (Fig. 1) as explanatory variables significantly explained 23.4 % (including Elsenborn) and 28 % (excluding Elsenborn) of the genetic variance (Table 6). None of the three least cost models (LCM10, LCM100 and LCM1000), having respectively five, three and five MEM-variables in the global model, yielded a significant overall result.

Discussion

Our results show that the roe deer populations sampled in Flanders display genetic diversity parameters that are slightly lower compared to the stable and large population from Elsenborn. Mean number of alleles, allelic richness and number of private alleles were higher in the Walloon population and comparable to other studies on roe deer using nearly the same set of markers (Coulon et al. 2004; Thulin 2006). The obtained genetic diversity parameters are also in the same range as those obtained for other ungulate species such as red deer in France (Dellicour et al. 2011), Belgium (Frantz et al. 2012), Germany (Kuehn et al. 2003) and Denmark (Nielsen et al. 2008) or moose in Norway (Haanes et al. 2011). Other estimates such as observed and expected heterozygosity were similar among all populations studied, except for Molenbeersel, which significantly differed from Elsenborn. Four Flemish populations (Schijnvallei, Meerdaal, Molenbeersel and Walenbos) nevertheless showed a significant heterozygosity deficit and deviation from Hardy-Weinberg equilibrium. However, when excluding the juveniles from the data set, Meerdaal no longer showed a deviation from HWE (data not shown). The deficit of heterozygotes

Table 4 Assignment of roe deer individuals to genetic clusters using STRUCTURE

Population	cluster 1	cluster 2	cluster 3	cluster 4
Bosbeekvallei	0.008	0.032	*0.917*	0.043
De Vart	0.003	0.006	0.023	*0.968*
Meerdaal	0.004	*0.887*	0.015	0.093
Molenbeersel	0.188	0.004	*0.615*	0.193
Balen	0.007	0.011	*0.456*	*0.526*
Walenbos	0.009	*0.351*	0.015	*0.626*
Elsenborn	*0.958*	0.007	0.027	0.007
Zwarte Beek	0.024	0.010	*0.794*	0.172
E19N	0.003	0.007	0.017	*0.973*
Schijnvallei	0.004	0.070	0.018	*0.908*

Assignment values higher than 30 % are indicated in *italic*

Table 5 Assignment of roe deer individuals to predefined populations using GENECLASS

	BBV	DV	MD	MB	B	WB	EB	ZB	EN	SV
Bosbeekvallei	*89.7*	0	2.6	2.6	2.6	0	0	2.6	0	0
De Vart	0	*90.6*	0	0	0	3.1	0	3.1	0	3.1
Meerdaal	2.1	0	*91.5*	0	0	2.1	0	2.1	2.1	0
Molenbeersel	3	0	0	*90.9*	0	0	0	6	0	0
Balen	9.1	9.1	0	9.1	59.1	4.5	0	9.1	0	0
Walenbos	0	0	0	3.6	0	*92.9*	0	0	0	3.6
Elsenborn	0	0	0	0	0	0	*98*	0	2	0
Zwarte Beek	6.1	0	3	6.1	3	0	3	*69.7*	6.1	3
E19N	0	6.7	0	0	0	0	0	0	*85.7*	6.7
Schijnvallei	0	13.5	2.7	0	0	8.1	0	0	5.4	*70.3*

Correct assignments of 70 % and higher are indicated in *italic*

Abbreviations are as in Table 2

may be either due to population substructure or inbreeding in a panmictic population. Within Flanders, there were also no significant differences in overall genetic diversity between the known relict populations (Molenbeersel and Meerdaal) and more recently established populations (E19N, Schijnvallei and Balen). The fact that we found no evidence for lower genetic diversity in more westwards, recently recolonized regions than in known relict populations, indicates that recolonization involved sufficient founding sizes, or that gene flow has already replenished the genetic diversity. Unreported translocations or release of captive animals may also have contributed.

Several of the roe deer populations examined show pairwise differentiation values above 0.08, indicating moderate to quite strong differentiation. The highest values are found between the relict and recent populations. This is reflected in a PCoA plot showing a smooth transition from south-east to north-west. Assignment tests using STRUCTURE and GeneClass also support the hypothesis of genetic differentiation and population structure in Flanders, although the power to uncover very recent genetic processes (<10 generations) with only ten genetic markers is rather low. STRUCTURE recognizes four main genetic clusters. From the perspective of ancient occurrence and current geographic distribution of roe deer in Flanders, a population structure with four clusters makes sense and might well correspond with the presence of remnant populations in the past and the recent recolonization, showing that expansion did not occur over large distances. This is in agreement with the general knowledge that roe deer do not disperse over large distances (Linnell et al. 1998), and that barriers may interrupt migration and gene flow (Coulon et al. 2006; Kuehn et al. 2007). The existence of a subtle population structure with little migration is further confirmed by the assignment tests with GeneClass which correctly reassigned a large part of the individual animals to the predefined populations except for Balen. This is not surprising as Balen is located in the middle of the study area and is more connected by suitable landscape with other adjacent sampled populations such as Zwarte Beek. As a result, gene flow among these centrally located populations might be higher. Three animals could not be assigned to any of the populations examined. It remains unclear from where they originate and whether they are natural migrants, especially since we cannot exclude the possibility of undocumented translocations.

Overall, our results point towards the existence of a quite clear local population structure at small spatial scale and rather restricted gene flow among the sampled units. To test whether this could be correlated with spatial distance, physical barriers or landscape features, we performed redundancy analyses. The RDA showed a significant and relatively strong effect of purely spatial structure (distance) that likely reflects spatial autocorrelation: nearby units are more similar than distant units. On the other hand, the position of populations relative to broad and major transportation infrastructure (two highways and a canal) had nearly as much explanatory power, while this model (consisting of just two binary variables) was much simpler. In contrast, the rather complicated least cost

Table 6 Results of the redundancy analyses using different explanatory models with (10 populations) or without (9 populations) Elsenborn

Expl. Var.	Pops	Var.	R^2_{adj}	Sel. Var.	R^2_{adj}
Space	9	4	0.302 (0.011)	2	0.302 (0.001)
	10	2	0.182 (0.023)	2	0.182 (0.023)
Roads/canals	9	2	0.280 (0.023)	2	0.280 (0.023)
	10	2	0.234 (0.010)	2	0.234 (0.010)
LCM10	9	5	NS (0.917)		
LCM100	9	3	NS (0.511)		
LCM1000	9	5	NS (0.990)		

Expl. Var. explanatory variant used in the model; *Pops* number of populations; *Var.* total number of variables in the global model; *Sel. Var.* number of selected variables; R^2_{adj} adjusted explained variance with the *p* value between brackets. Negative values are set to zero and are denoted as NS (not significant)

models had no explanatory power. Possibly, the fine-grained landscape structure, with a complex mosaic of both suitable and unsuitable patches, has no strong effect in our study approach. It is probable that such models are more appropriate for spatially-explicit, individual-based sampling designs at smaller spatial scales. Overall, our results suggest that the considered transportation infrastructure truly hinders gene flow. Individual-based studies have shown this elsewhere too for roe deer (Kuehn et al. 2007; Hepenstrick et al. 2012) or other large mammals (Perez-Espona et al. 2008; Frantz et al. 2012). Also, studies using telemetry showed that in the case of red deer, highways form a barrier that is hardly crossed (Licoppe 2006). In our study, the potential impact of anthropogenic barriers is further exemplified by the populations of Walenbos and Meerdaal, which despite their physical proximity, are relatively strongly differentiated from each other (F_{st}=0.072, D_{est}=0.083) and only partially belong to the same genetic cluster. Although it could not explicitly be tested, this may be due to the presence of the E40 highway and an accompanying railway separating the two populations from each other. In contrast, Balen and Zwarte Beek which are comparably close together, only show low differentiation levels (F_{st}=0.037, D_{est}=0.035), most probably because there are no harsh physical barriers separating them and there is sufficient suitable habitat in the landscape matrix between them. In this respect, it is unfortunate that we don't have individual coordinates of the animals' analyses. An individual-based approach would make it much more accurate to assess the impact of barriers at the one hand and for example green highway overpasses or corridors at the other hand on gene flow, especially in such a highly fragmented landscape as Flanders.

From this study, we cannot conclude that the twentieth century decline of roe deer, and its subsequent recovery and western recolonization, were accompanied by a noticeable reduction in genetic diversity. The spatially structured genetic diversity likely reflects the combined effect of spatial autocorrelation (isolation-by-distance) and major transportation infrastructure that reduces gene flow. As such, barriers and other landscape features (such as woodland) that influence dispersal have a genetic and biological significance that should be taken into account when defining management units. Rather than purely administrative boundaries, also genetic and other biological components should be taken into account for the delimitation of biological meaningful management units.

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Conflict of interest The authors declare that they have no conflict of interest. All experiments and collection of samples comply with the current Belgian laws.

References

Andren H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos 71:355–366

Antrop M (2004) Landscape change and the urbanization process in Europe. Landsc Urban Plan 67:9–26

Balkenhol N, Waits L (2009) Molecular road ecology: Exploring the potential of genetics for investigating transportation impacts on wildlife. Mol Ecol 18:4151–4164

Belkhir K (2004) Genetix 4.05.2. Laboratoire Génome et Populations, University of Montpellier II, Montpellier

Blanchet FGP, Legendre P, Borcard D (2008) Forward selection of explanatory variables. Ecology 89:2623–2632

Bright PW (1993) Habitat fragmentations; problems and predictions for British mammals. Mammal Rev 23:101–111

Cargnelutti B, Reby D, Desneux L, Angibault JM, Joachim J, Hewison AJM (2002) Space use by roe deer in a fragmented landscape. Some preliminary results. Rev Ecol (Terre Vie) 57:29–37

Casaer J (2003) Analysing roe deer habitat selection; methodological problems and possible solutions. *Dissertationes de agricultura*, 550. Faculteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen: Belgium. 197 pp

Casaer J, Licoppe A (2010) Ungulates and their management in Belgium, in: Apollonio, M. et al. (Ed.) (2010). European ungulates and their management in the 21st century. pp. 184–200

Coulon A, Cosson JF, Angibault JM, Cargnelutti B, Galan M, Morellet N, Petit E, Aulagnier S, Hewison AJM (2004) Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. Mol Ecol 13: 2841–2850

Coulon A, Guillot G, Cosson JF, Angibault JMA, Aulagnier S, Cargnelutti B, Galan M, Hewison AJM (2006) Genetic structure is influenced by landscape features: Empirical evidence from a roe deer population. Mol Ecol 15:1669–1679

Crawford N (2010) SMOGD: software for the measurement of genetic diversity. Mol Ecol Res 10:556–557

Deinet S, Ieronymidou C, McRae L, Burfield IJ, Foppen RP, Collen B, Böhm M (2013) *Wildlife comeback inEurope: The recovery of selected mammal and bird species.* Final report to Rewilding Europe by ZSL, BirdLife International and the European Bird Census Council. London, UK: ZSL

Dellicour S, Frantz AC, Colyn M, Bertouille S, Chaumont F, Flamand MC (2011) Population structure and genetic diversity of red deer (*Cervus elaphus*) in forest fragments in north-western France. Conserv Genet 12:1287–1297

Dray S, Legendre P, Peres-Neto PR (2006) Spatial modeling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol Model 196:483–493

Earl D, von Holdt B (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Epps CW, Palsboll PJ, Wehausen JD, Roderick GK, Ramey RR, McCullough DR (2005) Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. Ecol Lett 8:1029–1038

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annu Rev Ecol Evol Syst 34:487–515

Falush D, Stephens M, Pritchard J (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567–1587

Frantz A, Bertouille S, Eloy M, Licoppe A, Chaumont F, Flamand M (2012) Comparative landscape genetic analyses show a Belgian motorway to be a gene flow barrier for red deer (*Cervus elaphus*), but not wild boars (*Sus scrofa*). Mol Ecol 21:3445–3457

Galan M, Cosson JF, Aulagnier S, Maillard JC, Thevenon S, Hewison AJM (2003) Cross-amplification tests of ungulate primers in roe deer (*Capreolus capreolus*) to develop a multiplex panel of 12 microsatellite loci. Mol Ecol Not 3:142–146

Gilbert B, Bennett JR (2010) Partitioning variation in ecological communities: do the numbers add up? J Appl Ecol 47:1071–1082

Gobin A, Uljee I, Van Esch L, Engelen G, de Kok JL, Hens M, Van Daele T, Peymen J, Van Reeth W, Overloop S, Maes D (2009) Landgebruik in Vlaanderen : wetenschappelijk rapport - MIRA 2009 en NARA 2009. *Rapporten van het Instituut voor Natuur- en Bosonderzoek*, 2009(20). INBO: Brussel. ISBN 1782–9054. 328 pp

Goudet J (2001) FSTAT, A program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from www.unil.ch/izea/softwares/fstat.html

Haanes H, Roed KH, Perez-Espona S, Rosef O (2011) Low genetic variation support bottlenecks in Scandinavian red deer. Eur J Wildl Res 57:1137–1150

Hanski I (1998) Connecting the parameters of local extinction and metapopulation dynamics. Oikos 83:390–396

Hepenstrick D, Thiel D, Holderegger R, Gugerli F (2012) Genetic discontinuities in roe deer (*Capreolus capreolus*) coincide with fenced transportation infrastructure. Basic Appl Ecol 13:631–638

Hewison AJM, Vincent JP, Joachim J, Angibault JM, Cargnelutti B, Cibien C (2001) The effects of woodland fragmentation and human activity on roe deer distribution in agricultural landscapes. Can J Zool 79:679–689

Holderegger R, Wagner H (2008) Landscape genetics. Bioscience 58: 199–207

Jaeger JAG, Madrinan LF, Soukup T, Schwick C, Kienast F (2011) Landscape Fragmentation in Europe. Joint EEA-FOEN report. Pp 87

Jongman RHG (2002) Homogenisation and fragmentation of the European Landscape; ecological consequences and solutions. Landsc Urban Plan 58:211–221

Jost L (2008) G_{ST} and its relatives do not measure differentiation. Mol Ecol 17:4015–4026

Kuehn R, Schroeder W, Pirchner F, Rottmann O (2003) Genetic diversity, gene flow and drift in Bavarian red deer population (*Cervus elaphus*). Conserv Genet 4:157–166

Kuehn R, Hindenlang KE, Holzgang O, Senn J, Stoeckl B, Sperisen C (2007) Genetic effect of transportation infrastructure on roe deer populations (Capreolus capreolus). J Hered 98:13–22

Legendre P, Borcard D, Peres-Neto PR (2005) Analyzing beta diversity: Partitioning the spatial variation of community composition data. Ecol Monogr 75:435–450

Legendre P, Fortin MJ (2010) Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. Mol Ecol Res 10:831–844

Legendre P, De Caceres M, Borcard D (2010) Community surveys through space and time: Testing the space–time interaction in the absence of replication. Ecology 9:262–272

Legendre P, Legendre L (2012) Numerical Ecology. Third English edition. Elsevier Science BV, Amsterdam, The Netherlands

Licoppe A (2006) The diurnal habitat used by red deer (*Cervus elaphus* L.) in the Haute Ardenne. Eur J Wildl Res 52:164–170

Linnell J, Wahlström LK, Gaillard JM (1998) From birth to independence: birth, growth, neonatal mortality, hiding behaviour and dispersal. In: *The European Roe Deer: The Biology of Success* (eds Andersen R, Duncan P, Linnell JDC), pp. 257–283. Scandinavian University Press, Oslo, Norway

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: Combining landscape ecology and population genetics. Trends Ecol Evol 18:189–197

Morellet N, Van Moorter B, Cargnelutti B, Angibault JM, Lourtet B, Merlet J, Ladet S, Hewison AJM (2011) Landscape composition influences roe deer habitat selection at both home range and landscape scales. Landscape Ecol 26:999–1010

Nei M (1972) Genetic distances between populations. Am Nat 106:283–392

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Nielsen EK, Olesen CR, Pertoldi C, Gravlund P, Barker JSF, Mucci N, Randi E, Loeschcke V (2008) Genetic structure of the Danish red deer (Cervus elaphus). Biol J Linn Soc 95:688–701

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2011) Package 'vegan' version 2.0-2. http://cran.r-project.org/web/packages/vegan/index.html

Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol 13: 55–65

Perez-Espona S, Perez-Barberia FJ, Mcleod JE, Jiggins CD, Gordon IJ, Pemberton JM (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). Mol Ecol 17:981–996

Peres-Neto PR, Legendre P, Dray S, Borcard D (2006) Variation partitioning of species data matrices: Estimation and comparison of fractions. Ecology 87:2614–2625

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. J Hered 95:536–539

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

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci U S A 94:9197–9201

Rousset F (2008) Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Res 8:103–106

ter Braak CJF, Šmilauer P (2002) Canoco 4.5. Microcomputer power, Ithaca, New York

Thulin CG (2006) Microsatellite investigation of roe deer (*Capreolus capreolus*) in Scandinavia reveals genetic differentiation of a Baltic Sea Island population. Eur J Wildl Res 52:228–235

Tromcé M, Cahill S, De Vries JG, Farrall H, Folkeson L, Fry G, Hicks C, Peymen, J (Eds.) (2002) COST 341 - Habitat Fragmentation due to transportation infrastructure: The European Review, 253 pp. Office for Official Publications of the European Communities, Luxembourg

Vangestel C, Mergeay J, Dawson DA, Vandomme V, Lens L (2011) Genetic diversity and population structure in contemporary house sparrow populations along an urbanization gradient. Heredity 109: 163–172

Wahlström LK, Liberg O (1995) Contrasting dispersal patterns in two Scandinavian roe deer *Capreolus capreolus* populations. Wildl Biol 1:159–164

Wang M, Schreiber A (2001) The impact of habitat fragmentation and social structure on the population genetics of roe deer (*Capreolus capreolus* L.) in Central Europe. Heredity 86:703–715