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Genetic Effect of Transportation Infrastructure on Roe Deer Populations (*Capreolus capreolus*)

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Abstract

Anthropogenic transportation infrastructure is a major factor of habitat fragmentation leading to genetic population fragmentation in wildlife. Assessing and understanding the impact of this deterministic factor on genetic diversity and divergence of populations is crucial to appraise the viability of wildlife populations in fragmented landscapes. In this study, the roe deer is used as an example species for the assessment of genetic differentiation of populations separated by an anthropogenic barrier. In order to detect genetic discontinuities, we screened 12 polymorphic microsatellites on 222 individuals out of 11 roe deer populations that were sampled on the east and the westside of a fenced motorway in Central Switzerland. The interaction between landscape structure and microevolutionary processes such as gene flow and drift were assessed and evaluated by different population genetic methods like *F*-statistics, Mantel test, spatial autocorrelation analyses, Monmonier algorithm, and principal component analysis in conjunction with geographic information system data (synthesis map). We revealed an influence of the transportation infrastructure on genetic divergence of the roe deer population examined, but no impact on genetic diversity was detected. Based on the achieved genetic findings, recommendations for management implementation were made.

In Central Europe, transport infrastructure is present at a high density and thus contributes to landscape change and in particular to habitat fragmentation (Iuell et al. 2003). Habitat fragmentation caused by anthropogenic barriers can have negative demographic and genetic effects on wildlife populations (Reed et al. 2002). On the one hand, barriers lead to degradation, loss, and isolation of wildlife habitats (Ohmayer and Seiler 1985; Nellemann et al. 2001). On the other hand, for example, roads and railways are physical barriers and interrupt ecological processes such as dispersal and gene flow (Forman and Alexander 1998; Gerlach and Musolf 2000; Vos et al. 2001; Bhattacharya et al. 2003; Keller and Largiader 2003). Reduced gene flow can lead to small and isolated populations that are inherently more vulnerable to stochastic fluctuations and environmental perturbations (Frankham 1995; Keller and Waller 2002). Small and isolated populations are further threatened by inbreeding and loss of adaptive genetic variation (Randi 1993; Lande 1995; Hartl and Clark 1997). The quantitative assessment of the degree of isolation between local populations is therefore crucial to the appraisal of their viability.

The effects of roads on animal dispersal and movement have been documented in numerous studies and several determinants have been identified, such as road width, traffic volume, and animal behavior (for a review see Forman and Alexander 1998). Some empirical studies have shown the effects of anthropogenic barriers on genetic variation and gene flow in animal populations (e.g., Reh and Seitz 1990; Fowler et al. 2000; Gerlach and Musolf 2000; Keller and Largiader 2003; Edwards et al. 2004; Keller et al. 2004, 2005). The partitioning of genetic variation within and between local populations is primarily determined by the dynamic balance between gene flow and local genetic drift (Wright 1943, 1982). Accordingly, reduced gene flow due to the barrier effect of roads is expected to lead to an increase of population differentiation and to a decrease of genetic diversity within

local populations exposed to prolonged isolation. This assumption is supported by the following studies: for small and less mobile species such as the ground beetle (*Carabus violaceus*, *Abax parallelepipedus*), the common frog (*Rana temporaria*), or the desert tortoise (*Gopherus agassizii*) motorways may be absolute barriers to gene flow leading to a loss of genetic variation and possibly to the extinction of local populations (Reh and Seitz 1990; Keller and Largiader 2003; Edwards et al. 2004). Gerlach and Musolf (2000) demonstrated significant population subdivision in bank vole (*Clethrionomys glareorus*) populations separated by a motorway, but no effect of smaller roads on population structure could be found. Other studies revealed decreased levels of genetic diversity within local populations surrounded by roads and other human-made barriers (Reh and Seitz 1990; Keller and Waller 2002; Epps et al. 2005; Keller et al. 2005). Although most of these studies exemplify the genetic effects of roads on small animals, information on larger European mammals with higher dispersal capacity is not available yet.

In the present study, we analyze the effect of transportation infrastructure on the genetic structure of European roe deer (*Capreolus capreolus*) populations. The roe deer is a medium-sized ungulate with high ecological and behavioral plasticity inhabiting a variety of environments, including forests, shrub lands, and marshes (Linnell, Duncan, and Andersen 1998). In fragmented landscapes, roe deer is mainly confined to wooded environments showing high site fidelity (Hewison et al. 2001). Dispersal primarily occurs in yearlings, whereas females and males have different spatial strategies (Wahlstrom 1994; Wahlstrom and Kjellander 1995; Müri 1999). Rate and distance of dispersal greatly vary among regions and are related to habitat quality and habitat structure (Linnell, Wahlström, and Gaillard 1998; Müri 1999). In Central Europe, dispersal rates of yearlings range from 20% to 75% and dispersal distances rarely exceed a few kilometers (Stubbe 1997; Linnell, Wahlström, and Gaillard 1998; Müri 1999). Such a pattern of limited dispersal is expected to result in population differentiation increasing with geographic distance in undisturbed roe deer populations. This isolation-by-distance effect is overruled in common European landscapes, where habitat fragmentation leads to reduced gene flow and influences the genetic structure of roe deer populations due to agricultural practices or fragmented woodland (Coulon et al. 2004). In highly fragmented habitats, Wang and Schreiber (2001) found genetic distances between adjacent roe deer populations to correlate with urbanization and not with distance. Thus, there is a good reason to assume that transportation infrastructure not permeable to roe deer such as fenced motorways form sharp genetic barriers and lead to differentiated populations.

To verify the influence of anthropogenic barriers on gene flow between local roe deer populations, we analyzed samples from 8 locations along a motorway in the region of Sursee, Switzerland. The aim was to reveal 1) whether transportation infrastructure leads to a spatial genetic pattern of roe deer populations and 2) whether transportation infrastructure affects genetic diversity of roe deer. To compare the results with diversity patterns on a larger geographic scale, we included 3 populations of the region around Zurich that are separated by a lake.

The detection of genetic discontinuities and their correlation with landscape and environmental features are the key steps of landscape genetics (Manel et al. 2003). The analyses of interaction between landscape features and microevolutionary processes, such as gene flow and drift, are of high relevance regarding the restoration of wildlife migration corridors in anthropogenic-fragmented landscapes.

Material and Methods

Study Site and Sampling Design

Our study site is located in Switzerland in the area of Sursee (Figure 1). It covers approximately 24 × 16 km and is composed of a mosaic of managed forests and agricultural land, fragmented by a motorway, major roads, railways, and villages. The fenced motorway is known to interrupt several wildlife migration corridors that are of national relevance (Holzgang et al. 2001). The motorway was constructed in 1980 and opened to traffic in 1981. Along the 28 km of the motorway, there are 11 overpasses and 3 underpasses constructed for local transport (Table 1).

Roe deer populations were sampled by hunters in 1998 and 2000 in 8 forest patches, named S1W, S2W, S3W, and S4W on the westside of the motorway and S1E, S2E, S3E, and S4E on the eastside of the motorway (Figure 1). Equal numbers indicate pairs of sampled populations separated by the motorway. Characteristics of the barriers separating these sampling units are given in Table 1. A total of 136 roe deer was sampled (68 females and 68 males).

To assess the levels of population differentiation in the Sursee region in comparison to populations in a distance of approximately 80 km, 3 additional roe deer populations were sampled in the area of the lake of Zurich in 2000 and 2001 (Figure 1). Z1 and Z2 are divided by an urban area and a major road and Z3 is separated from Z1 and Z2 by the lake of Zurich. In this area, 86 roe deer (57 females and 29 males) were sampled.

From each individual, geographic information system data of the location were recorded and heart tissue (20 g) was collected and stored at −20 °C.

DNA Isolation and Microsatellite Analyses

DNA was extracted from heart tissue according to Hogan et al. (1986) and genotyped for 12 microsatellite loci derived from cattle, sheep, and roe deer (Table 2). The microsatellites were selected based on information of the Deer Genetic Linkage Map (Slate et al. 2002) and on the level of polymorphisms identified for deer populations (Kuehn et al. 1996, 2003; Vial et al. 2003). The microsatellites were amplified by PCR in 15 μl composed of 40–60 ng DNA, 1× PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.5–3.0 mM $MgCl_2$ (depending on the primer pairs as given in Table 2), 0.1 mM dNTPs, 0.2 μM of each primer and 0.3 units of Taq

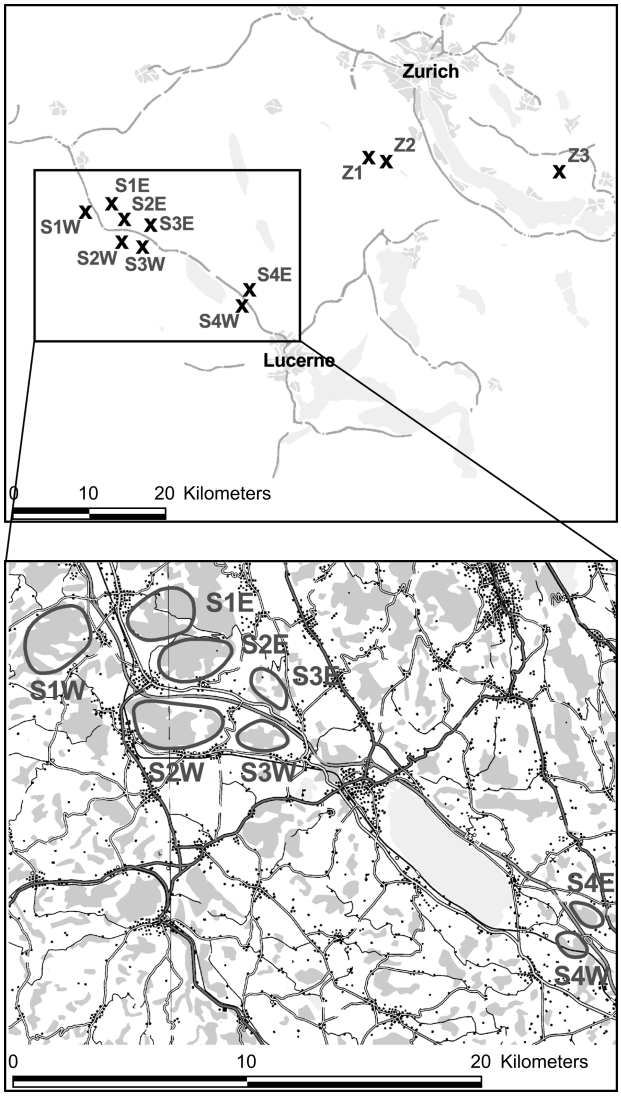

Figure 1. Map showing the sampled roe deer populations in the region of Zurich (Z1–Z3) and Sursee (S1W–S4W west of the motorway, S1E–S4E east of the motorway). Dark gray indicates managed forests.

DNA polymerase (MBI-Fermentas, St Leon-Rot, Germany). We used the following PCR profile: initial denaturation at 94 °C for 3 min, 35 cycles consisting of 45 s denaturation at 94 °C, 45 s annealing at temperatures given in Table 2, 45 s extension at 72 °C, followed by final extension at 72 °C for 3 min. Thermal cycling was performed in a BIOMETRA UNO cycler. The forward primers were end-labeled with the fluorescent dye Cy5, and the length of the microsatellite alleles was determined on an ALF-EXPRESS II DNA analyzer (Amersham Pharmacia Biotech, Munich, Germany) and ALLELELINKS version 1.02 software (Amersham Pharmacia Biotech). To ensure consistent scoring of genotypes within and among gels, we included internal size standards in each lane and known genotypes on each gel. The proportion of missing genotypes was below 5% per locus.

Measures of Genetic Variation

To assess genetic variation within populations, we used the following measures: average number of alleles per locus, allelic richness, and expected and observed heterozygosity. All measures were calculated for each locus with FSTAT version 2.9.2 (Goudet 2001). Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were estimated in GENEPOP version 3.3 (Raymond and Rousset 1995b) with probability tests carried out with 100 000 iterations in the Markov chain method after 1000 burn-in steps (Guo and Thompson 1992; Raymond and Rousset 1995a). In order to correct for effects caused by multiple testing, we applied sequential Bonferroni adjustments (Rice 1989). As an additional measure of within-population variation, relatedness between individuals was analyzed for each locus based on F values (Ciofi and Bruford 1999). The parameter F refers to the probability that 2 genes share a common ancestor and was obtained from 2MOD (Ciofi and Bruford 1999). A Markov chain Monte Carlo simulation with 1 000 000 iterations was computed, and the first 10% of the output was discarded in order to avoid bias due to the starting conditions.

Variation between populations was estimated by the F_{ST} value (Weir and Cockerham 1984). Tests for significant population differentiation among all pairs of populations were performed with GENEPOP using 100 000 iterations in the Markov chain after 1000 burn-in steps (Raymond and Rousset 1995b).

To determine how total genetic variance is partitioned into covariance components at different hierarchical levels, we performed an analysis of molecular variance (AMOVA) with Arlequin version 2.000 (Schneider et al. 2000). The following 3 hierarchical levels were analyzed: 1) within individuals, 2) among individuals within populations, and 3) among populations. Based on the covariance components, the fixation

Table 1. Characteristics of barriers separate pairs of populations in the region of Sursee, Switzerland

Population pair	Populations west of motorway	Populations east of motorway	Barrier characteristics	Over- and underpasses for local transport
I	S1W	S1E	Fenced motorway, 2 regional roads, railway line, densely populated area	2 overpasses
II	S2W	S2E	Fenced motorway, regional road	4 overpasses and 1 underpass
III	S3W	S3E	Fenced motorway, regional road, sparsely populated area	2 overpasses
IV	S4W	S4E	Fenced motorway, railway line	3 overpasses and 2 underpasses

Table 2. Characterization of the analyzed microsatellite loci in roe deer (*C. capreolus*). Locus and groupings used in multiplex gels, primer sequences, annealing temperature T_a, $MgCl_2$ in millimolars, number of observed alleles (N_A), range of allele sizes and average expected (H_e) and observed (H_o) heterozygosity, results of probability tests for deviation from expected Hardy–Weinberg expectations (P_{HW})

Locus	Multiplex	PCR primers	T_a	$MgCl_2$	N_A	Allele size (bp)	H_e	H_o	P_{HW}
BM1818[a]	I	F: AGTGCTTTCAAGGTCCATGC, R: AGCTGGGAATATAACCAAAGG	60	3.0	8	246–262	0.746	0.720	ns
CSSM06[b]	II	F: AGCTTCTGACCTTTAAAGAAAATG, R: AGCTTATAGATTTGCACAAGTGCC	57	3.0	2	188–190	0.448	0.442	ns
CSSM19[b]	III	F: TTGTCAGCAACTTCTTGTATCTTT, R: TGTTTTAAGCCACCCAATTATTTG	55	3.0	2	126–128	0.214	0.243	ns
CSSM22[b]	III	F: TCTCTCTAATGGAGTTGGTTTTTG, R: ATATCCCACTGAGGATAAGAATTC	53	3.0	2	211–213	0.056	0.053	ns
ETH225[c]	IV	F: GATCACCTTGCCACTATTTCCT, R: ACATGACAGCCAGCTGCTACT	53	1.5	5	138–146	0.705	0.587	ns
INRA35[a]	IV	F: ATCCTTTGCAGCCTCCACATTG, R: TTGTGCTTTATGACACTATCCG	55	3.0	4	95–98	0.235	0.218	ns
MAF70[d]	II	F: CACGGAGTCACAAAGAGTCAGACC, R: GCAGGACTCTACGGGGCCTTTGC	63	1.5	12	117–159	0.789	0.793	ns
MM12[b]	V	F: CAAGACAGGTGTTTCAATCT, R: ATCGACTCTGGGGATGATGT	53	3.0	2	83–87	0.040	0.030	ns
NVHRT16[e]	VI	F: ATTCTAAGCCCAAATAATCTT, R: TCTAAGGGGTCTGTGTCTT	55	1.5	10	158–182	0.787	0.817	ns
NVHRT24[e]	VI	F: TGTGGACTATAGGGAGC, R: GTGTACAAAAAGTGATTGAGT	55	1.5	8	130–148	0.764	0.800	ns
OarFCB304[d]	VII	F: CCCTAGGAGCTTTCAATAAAGAATCGG, R: CGCTGCTGTCAACTGGGTCAGGG	57	3.0	12	164–186	0.820	0.741	ns
Roe01[f]	VII	F: AAATTTGGCTCTGCAATCGG, R: ACACAAAAGCCACCCAATAC	53	1.5	2	131–133	0.454	0.524	ns

P_{HW} significant ($P < 0.0045$) (Bonferroni correction).

[a] Steffen et al. (1993).

[b] Moore et al. (1994).

[c] Bishop et al. (1994).

[d] Buchanan and Crawford (1992).

[e] Roed and Midthjell (1998).

[f] Fickel and Reinsch (2000).

indices F_{IS}, F_{IT}, and F_{ST} were calculated and tested for their significance.

Spatial Analyses

We used 4 approaches to characterize spatial patterns of genetic variation. First, Mantel tests were performed to evaluate the impact of geographic distance on population differentiation. Geographic distances between sampling sites were calculated based on the coordinates of the approximate center of the sampling areas. Correlations between geographic distances and genetic distances $[F_{ST}/(1 - F_{ST})]$ (Rousset 1997) were calculated with the R-PACKAGE-module Mantel (Casgrain 2001). The statistical significance of the relationships was determined with 10 000 randomizations. These tests were carried out with and without the samples of the Zurich area.

Second, we carried out spatial autocorrelation analyses to determine to which extent, as measured in geographic distance, patterns of genetic variation of neighboring samples are associated (Epperson 2003). We used an approach developed for multiallelic codominant markers (Smouse and Peakall 1999), implemented in GenAlEx version 5.0 (Smouse and Peakall 1999). These analyses were based on data from individuals. We used the square genetic distances between individuals (Smouse and Peakall 1999), their geographic distances calculated from coordinates where each roe deer was shot, and even distance classes of 8 km. The autocorrelation index r of each distance class was statistically tested by 1000 random permutations providing a 95% confidence interval. The autocorrelation coefficient r is bounded by $[-1, +1]$ and is closely related to Moran's I-value. A significant positive value of r indicates that the pairs of individuals within a given distance class have more alleles in common than would be expected by chance, whereas a significant negative value indicates that such individuals have fewer alleles in common than expected. Nonsignificant r values indicate a random distribution of genotypes within a distance class. The autocorrelation analyses were carried out with and without the samples of the Zurich area.

Third, we applied the Monmonier algorithm (Monmonier 1973) that identifies genetic boundaries, that is zones where genetic differences between pairs of populations are highest. This approach is suitable to detect abrupt genetic changes, as it is expected in the Sursee area due to the motorway. Using

Table 3. Microsatellite diversity indices of roe deer (*C. capreolus*) populations of the Zurich and Sursee region, Switzerland. Sample size (N), average number of alleles/locus (A), mean allelic richness (A_R), expected (H_e) and observed (H_o) heterozygosity, result of Hardy–Weinberg probability test for deviation from expected Hardy–Weinberg proportions (P_{HW}) using the FSTAT program (Goudet 2001) and the F values based on the 2MOD program (Ciofi and Bruford 1999)

Population	N	A	A_R	H_e	H_o	P_{HW}	F
Sursee populations							
S1W	24	4.3	3.48	0.526	0.504	ns	0.0452
S1E	29	4.3	3.48	0.512	0.494	ns	0.0166
S2W	17	3.6	3.18	0.503	0.539	ns	0.0598
S2E	25	4.3	3.61	0.510	0.486	ns	0.0129
S3W	8	3.6	3.58	0.497	0.490	ns	0.0199
S3E	11	4.2	3.83	0.501	0.477	ns	0.0087
S4W	12	3.9	3.59	0.532	0.525	ns	0.0219
S4E	10	3.6	3.41	0.471	0.495	ns	0.0350
Zurich outgroups							
Z1	31	4.7	3.63	0.515	0.505	ns	0.0347
Z2	22	4.3	3.54	0.506	0.494	ns	0.0441
Z3	33	4.3	3.36	0.481	0.464	ns	0.0539

P_{HW} significant ($P < 0.0042$) (Bonferroni correction).

BARRIER version 2.2 (Manni et al. 2004), populations of the Sursee area were connected by a Delaunay triangulation (Brassel and Reif 1979), followed by the application of the Monmonier's maximum difference algorithm. To assess the robustness of computed barriers, 100 resampled bootstrap F_{ST} matrices over all pairs of the Sursee populations were calculated.

Fourth, we generated a synthesis map with the first principal component (PC) scores obtained from a PC analysis using allele frequencies of each locus and each population as variables. PC1 scores were interpolated within space by the kriging interpolation procedure (Journel and Huijbregts 1978). The interpolated lines represent zones of equal genetic divergence (isogene). The number of isogenes between 2 sampling sites shows the degree of their genetic differentiation. This approach complements the Monmonier analysis and allows to identify zones of reduced gene flow. The analyses were carried out with STATISTICA 6.0 and SURFACE III software (Kansas Geological Survey) according to Piertney et al. (1998).

Results

Linkage and Hardy–Weinberg Equilibrium

Of the 66 tests for linkage disequilibrium across all populations, one was significant ($P < 0.05$). Three tests are expected to be significant at the $\alpha = 0.05$ level by chance. Within populations, no significant values were obtained. Thus, there was no evidence for linkage among the 12 microsatellite loci. Genotypic frequencies generally conformed to Hardy–Weinberg expectations (Tables 2 and 3).

Genetic Diversity and Relatedness within Populations

The 12 microsatellite loci showed 2 to 12 alleles with a mean of 5.8 alleles per locus (Table 2). Within populations, the mean number of alleles per locus ranged from 3.6 to 4.7 and allelic richness from 3.18 to 3.83 (Table 3). Observed heterozygosity ranged from 0.464 to 0.539 and expected heterozygosity from 0.471 to 0.532. The number of individuals was positively correlated with the mean number of alleles per locus A ($r^2 = 0.857$, $P < 0.001$), but not significantly with allelic richness A_R ($r^2 = -0.100$, $P > 0.7$), expected heterozygosity H_e ($r^2 = 0.327$, $P > 0.3$), and observed heterozygosity H_o ($r^2 = -0.123$, $P > 0.7$). Thus, the genetic parameters A_R, H_e, and H_o were not biased by differences in sample size. No significant differences with respect to A, A_R, H_e, and H_o between the analyzed populations were detectable based on t-tests.

The proportion of common ancestors within each population, as inferred from the F values was lowest in S3E ($F = 0.0087$) and highest in S2W ($F = 0.0598$). Overall, the probability that 2 genes share a common ancestor is low. Generally, the findings of genetic diversity and relatedness point to a high genetic variability within the roe deer populations examined.

Genetic Differentiation and Spatial Analyses

Thirty-six of the 55 pairwise estimates of F_{ST} were significant (Table 4). In the Sursee area, 9 of the 28 estimates were significant. The highest F_{ST} values were observed between the populations from the Zurich and from the Sursee area. Within the Sursee area, the F_{ST} values of populations separated by the motorway were generally higher than those between populations on either side of the motorway (t-test, $P = 0.007$, see Table 4, first diagonal vs., second diagonal with the exception of the value between S1W-S2W and S3E-S3W). Additionally, S1W showed the highest F_{ST} values compared with all other populations along the motorway revealing an isolated population structure. The F_{ST} value between S3E and S3W was negative indicating gene flow between these 2 populations. The genetic differentiation between populations separated by the anthropogenic transportation infrastructure was slightly lower ($\Delta F_{ST} = 0.01$, with the exception

Table 4. Matrix of the F_{ST} values according to Weir and Cockerham (1984) between roe deer populations. In bold are the pairwise comparisons between populations of opposite sides of different anthropogenic and natural barriers

	S1E	S1W	S2E	S2W	S3E	S3W	S4E	S4W	Z1	Z2	Z3
S1E											
S1W	**0.0161***										
S2E	0.0044	0.0301***									
S2W	0.0141**	0.0313***	**0.0087***								
S3E	0.0016	0.0105	−0.0116	0.0058							
S3W	0.0013	0.0331*	−0.0114	0.0077	**−0.0216**						
S4E	0.0117	0.0213*	0.0066	0.0203	−0.015	0.0118					
S4W	0.0066	0.0202*	0.0057	0	0.002	−0.014	**0.0154****				
Z1	0.0124*	0.0369***	0.0375***	0.0372***	0.0311*	0.0444*	0.0591**	0.0442***			
Z2	0.0329***	0.0557***	0.0520***	0.0405***	0.0354*	0.0528*	0.0759**	0.0505*	0.0080*		
Z3	0.0179***	0.0429***	0.0351***	0.0353***	0.0290*	0.0523***	0.0536***	0.0425***	0.0194***	**0.0303****	

Significant value for population allelic differentiation: $P > 0.05$; * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$.

of S3E-S3W) than the differentiation between populations separated by the natural barrier in the Zurich area (Table 4).

The AMOVA showed that 96.2% of the variance is explained by within-individual variation, 1.2% by variation between individuals within population, and 2.6% by variation between populations. Although the total genetic variation accounted mainly for within-individual variation, there was twice as much variation between populations than between individuals within populations, indicating a certain population structure. The overall *F*-statistics revealed significant values for $F_{IS} = 0.0126$ ($P < 0.01$), $F_{IT} = 0.0262$ ($P < 0.001$), and $F_{ST} = 0.0385$ ($P < 0.0001$). Wright (1978) identified the problem of interpreting F_{ST} values as an absolute value based on highly polymorphic loci and proposed that a $F_{ST} < 0.05$ could indicate a considerable population differentiation.

A significant correlation between genetic and geographic distances was observed ($r^2 = 0.215$, $P < 0.01$) including all 11 populations in the Mantel analysis. In contrast, there was no evidence for an isolation-by-distance effect in the Sursee area ($r^2 = 0.052$, $P > 0.05$). The discrepancy of these 2 analyses is likely based on the long distance effect of the Zurich populations. The spatial autocorrelation analysis using individual data including all sampled individuals shows significant positive *r* values for the distance class of 8 km and significant negative values for the distance classes of 48, 56, 64, and 88 km (Figure 2A). The overall slope of this correlogram was significantly negative ($\beta = -0.0045$, $P = 0.009$, $r^2 = 0.528$), indicating a weak pattern of spatial autocorrelation. However, analyzing only the Sursee individuals no significant *r* values resulted for all distance classes (Figure 2B) and there was no overall slope detectable ($\beta = -0.0000$, $P = 0.992$, $r^2 = 0.000$). These results point at the absence of an isolation-by-distance effect in the Sursee area and indicate a population fragmentation effect due to the anthropogenic barriers in the region.

To identify the exact locations where population subdivision occurs in the Sursee area, we used 2 complementary approaches. Using the Monmonier algorithm, we identified 3 barriers showing a constant decrease from higher to lower genetic distances (Figure 3). The first barrier separates population S1W from the surrounding populations S2W and S1E—both population pairs showing high bootstrap values (≥94%). The second barrier separates population S2W from the populations S1E, S2E, and S3W, whereas the population pair S2W and S1E shows the highest bootstrap value (94%). Finally, populations S4W and S4E are separated with a bootstrap result of 62%.

The contours of the first-axis PC scores plotted on the population centers and interpolated on the area delimiting the populations of the Sursee area show a similar picture (Figure 4). The synthesis map reveals considerable genetic

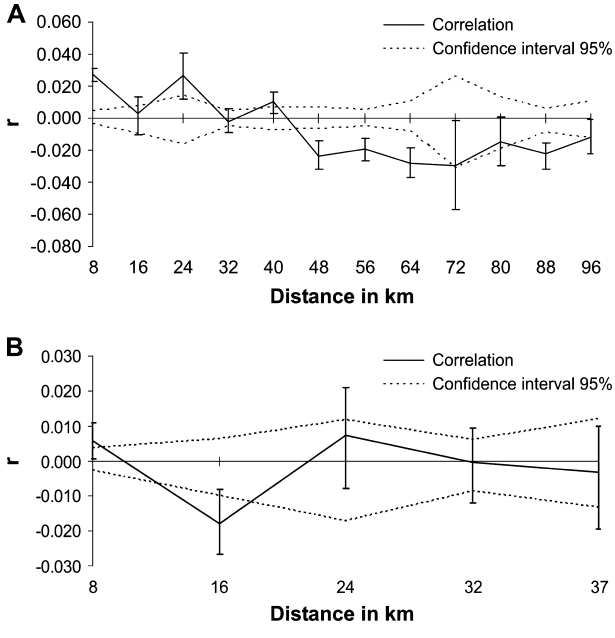

Figure 2. Spatial autocorrelogram showing the combined genetic correlation (*r*) as a function of distance for (**A**) all sampled individuals and (**B**) for the Sursee individuals. The 95% confidence interval of the null hypothesis assuming a random distribution of genotypes (dashed lines) and standard deviation of *r* determined by bootstrapping (bars) are shown.

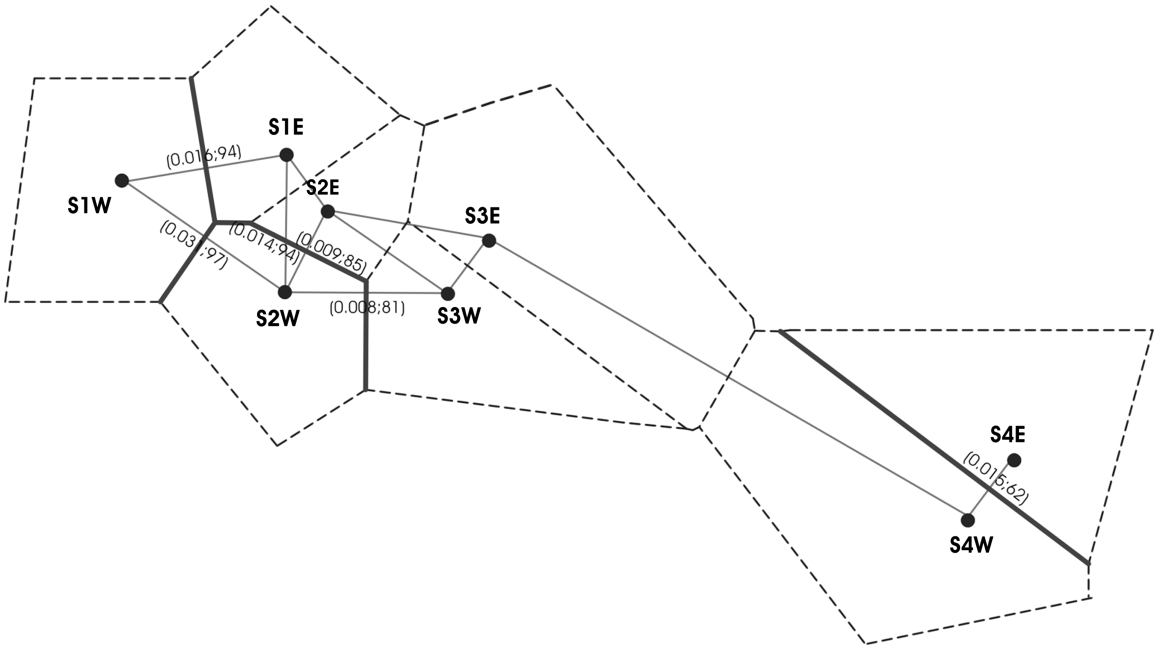

Figure 3. Detection of barriers (bold lines) to gene flow between the 8 roe deer populations in the Sursee region using the Monmonier algorithm (Monmonier 1973). Dots indicate the geographic localities of the populations, fine lines show the connections of localities based on the Delaunay triangulation (Brassel and Reif 1979). Dashed lines indicate the Voronoi (1908) tessellation. The F_{ST} and bootstrap values are shown in brackets closed to the barriers.

divergence between populations east and west of the motorway, especially for the population pairs S1W and S1E and S1W and S2E as well as the pairs S2W and S1E and S2W and S2E. However, the populations S3E and S3W as well as S4E and S4W show substantial gene flow.

Discussion

The results of our study allow for 2 main conclusions: 1) Anthropogenic transportation infrastructure represents a barrier to gene flow between local roe deer populations leading to population differentiation. 2) The observed fragmentation of populations does not affect genetic diversity within populations.

Genetic Variability within Populations

By applying 12 microsatellites, we were able to detect comparable values of expected heterozygosity and allelic richness in all of the roe deer populations examined. These results are consistent with previous studies on the genetic structure of

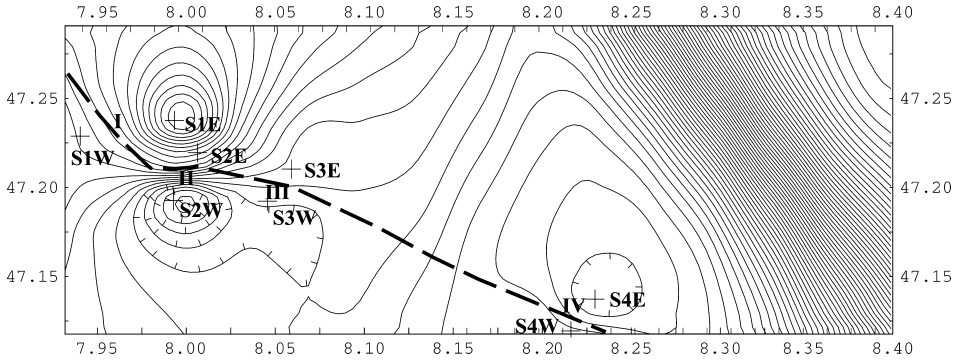

Figure 4. Synthesis map showing the hypothetical first-axis PC1 scores derived from the kriging procedure in 2 dimensions for the Sursee populations. The lines connect points of equal PC1 scores (isogene). The sampling locations (populations S1W–S4W west of the motorway, S1E–S4E east of the motorway) and the examined population pairs I–IV are highlighted. The motorway is schematically shown as a dashed line.

European roe deer (Postma et al. 2001; Wang and Schreiber 2001; Randi et al. 2004) and other ungulate species (Forbes et al. 1995; Barker et al. 1997; Kuehn et al. 2003, 2004). We found no evidence for a reduced genetic diversity within the analyzed populations (H_e: Friedman analysis of variance (ANOVA), $P = 0.49$; A_R: Friedman ANOVA, $P = 0.38$) reported for other animal species suffering from fragmentation (*Ovis canadensis nelsoni* [Epps et al. 2005], *C. violaceus* [Keller and Largiader 2003], *Ursus arctos* [Randi 1993], *Crotaphytus collaris*). One reason may be that the isolation due to the 20-year-old motorway did not last long enough to cause reduced genetic diversity within the fragmented populations. Another reason could be that there is a noteworthy gene flow between the populations examined and further distant roe deer populations on either side of the motorway. Especially, the high genetic diversity of the segregated population S1W points to considerable gene flow between this and neighboring (unanalyzed) populations in the backcountry. A high genetic variability within the populations and the lack of severe isolation effects can be considered in general for the roe deer populations examined.

Genetic Variation between Populations and Effect of the Anthropogenic Barriers

Nevertheless, the analyses of genetic variation between the 11 roe deer populations examined revealed an apparent fragmented population structure. Even though most of the genetic variance is explained by within-individual variation, the genetic variance explained by variation between populations is 2 times higher than the one given by variation between individuals within a population. Population differentiation can result from 2 genetic processes. 1) Population differentiation may increase with geographic distance because the influence of gene flow relative to genetic drift declines with geographic distance. 2) Populations may be fragmented and thus gene flow is reduced among local populations.

In this study, we found a spatial structure of genetic variation that is shaped by effects of distance and anthropogenic fragmentation. As expected because of the generally limited dispersal of European roe deer (Stubbe 1997; Linnell, Wahlström, and Gaillard 1998; Müri 1999), genetic differentiation caused by isolation-by-distance can be observed when including all 11 populations in the correlation analyses. Also highest values of differentiation (F_{ST}) can be found between the 3 populations in the Zurich region and the 8 populations in the Sursee region separated by approximately 80 km. These results are consistent with other studies of ungulates that found evidence for a genetic population structure related to geographic distance in accordance to Wrights' (1943) model of isolation-by-distance (Wang and Schreiber 2001; Edwards et al. 2004; Nies et al. 2005).

In contrast, no isolation-by-distance effect could be detected between the populations in the Sursee region as shown by the results of the Mantel test and the spatial autocorrelation analysis. Additionally, population separated by the motorway showed higher genetic differentiation than population on either side of the road. Furthermore, the boundaries of gene flow identified by the Monmonier algorithm and the synthesis map coincide with the geographic position of the motorway. We conclude that roe deer disperse between the populations on the same side of the motorway, but that movement and therefore gene flow are prevented across the fenced motorway. The barrier effect of roads to gene flow has clearly been shown for small mammal species such as voles and ground beetles (Gerlach and Musolf 2000; Keller et al. 2004). In a recent study, Epps et al. (2005) reported the elimination of gene flow between populations of desert bighorn sheep (*Ovis canadensis nelsoni*) due to human-made barriers such as highways and developed areas existing only for 40 years. They concluded that anthropogenic barriers constitute a severe threat to the persistence of naturally fragmented populations being the case in desert bighorn sheep. In contrast, naturally fragmented populations probably not occur in roe deer because levels of genetic differentiation are generally low throughout Central Europe (Wang and Schreiber 2001).

However, gene flow is not restricted across all parts of the motorway in our study: genetic differentiation is nonexistent between the 2 populations S3W and S3E. Presumably, the overpass between the 2 locations is being used for crossing the motorway by roe deer. In contrast to all other existing over- and underpasses that were erected as local transport links and connect villages and agricultural fields, the overpass between S3W and S3E connects 2 mainly forested areas. Thus, roe deer dispersal and the use of possible migration corridors across the motorway seem strongly linked to wooded structures. Also, Hewison et al. (2001) and Coulon et al. (2004) emphasized the importance of woodland for roe deer dispersal.

In addition, other factors apart from the motorway seem to influence the genetic structure of roe deer populations in the Sursee region. S1W is highly differentiated from all other populations on the east and the westside of the motorway. This may be caused by one of the following reasons: first, the population has not reached its genetic equilibrium yet because of historical and other anthropogenic disturbances (e.g., hunting). However, due to the high genetic diversity within the S1W population, this hypothesis is unlikely. A second and more probable explanation is that dispersal to S2W is reduced due to fragmentation caused by a major road and settlements leading to an isolated population structure of S1W. Also, Epps et al. (2005) emphasized that other human-made barriers other than highways such as canals and developed areas block gene flow between neighboring populations.

Overall, our analyses show that human-made barriers constructed less than 20 years ago create a detectable increase of genetic differentiation of the roe deer populations examined. However, this effect caused by an anthropogenic barrier is slightly lower compared with the effect of the natural barrier, the lake of Zurich between Z1/Z2 and Z3. Our study also points to the conclusion that the continuance and permeability of barriers influence the genetic differentiation of populations as revealed by the genetic isolation of S1W and the linkup between S3W and S3E.

Comparing the different statistical analyses, this study shows that especially the Monmonier algorithm and the synthesis map, which enable the visualization of spatial genetic patterns within a geographic map, are powerful molecular genetic tools to delineate the localization of the genetic boundaries.

Implications for Conservation and Management

Our results demonstrate that genetic analyses allow for conclusions on landscape-relevant issues, such as the effect of anthropogenic barriers on animal populations and their migration routes. The ongoing expansion of roads, urban settlements, and other barriers will further cause reduced and fragmented habitat for many species. Solutions to restore wildlife corridors and therefore landscape connectivity have to be implemented (e.g., road planning). Overpasses and green bridges constructed for wildlife represent a counter measure to barriers such as motorways (Holzgang et al. 2001, 2005; Forman et al. 2003; Iuell et al. 2003). Genetic analyses can contribute to reveal the most appropriate location of an overpass in order to connect genetically isolated wildlife populations. In addition, genetic data can help to evaluate the effect of such counter measures on the restoration of dispersal between animal populations and allow a quantitative assessment of the viability of the populations in concern.

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