The significance of major roads as barriers and their roadside habitats as potential corridors for hazel dormouse migration – a population genetic study

Authors: Friebe, Kathrin, Steffens, Thomas, Schulz, Björn, Valqui, Juan, Reck, Heinrich, et al.

Source: Folia Zoologica, 67(2) : 98-109

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.v67.i2.a10.2018

Folia Zool. – 67 (2): 98–109 (2018) DOI: 10.25225/fozo.v67.i2.a10.2018

The significance of major roads as barriers and their roadside habitats as potential corridors for hazel dormouse migration – a population genetic study

Kathrin FRIEBE[1], Thomas STEFFENS[1], Björn SCHULZ[2], Juan VALQUI[1], Heinrich RECK[1] and Georg HARTL[1]

[1] *University of Kiel, Institute for Natural Resource Conservation, Olshausenstrasse 75, Kiel, Schleswig-Holstein D-24118, Germany*
[2] *Stiftung Naturschutz Schleswig-Holstein, Eschenbrook 4, Molfsee D-24113, Germany; e-mail: schulz@sn-sh.de*

Received 24 January 2018; Accepted 21 August 2018

Abstract. Major roads are commonly regarded as migration barriers for most terrestrial animal species. Hazel dormouse, *Muscardinus avellanarius*, populations in south-eastern Schleswig-Holstein were investigated in order to assess the possible effects of major roads on their genetic variability and genetic structure. A total of 177 samples were collected and analysed, using ten microsatellite loci. Estimates of genetic diversity (expected heterozygosity: 0.48-0.65, allelic richness: 2.9-3.9) were within the range commonly found in this species. No evidence of inbreeding or past bottlenecks was detected. The software structure grouped the samples into five subpopulations. However, this subdivision should be treated with caution, since many individuals with mixed or unclear genetic profiles were found, possibly representing migrants or their offspring. Contrary to the previous assumption that dormice hardly ever cross roads, the present study shows that dormice not only cross even major roads, but also hold close relationships to individuals living on the other side of the motorway. The high number of animals captured within a small area and the relatively low genetic differentiation (F_{ST}: 0.142 and 0.105) despite the great distances (33.1 and 25.6 km) along the road, indicate that the roadside shrubs can actually be good habitats for dormice and provide suitable corridors for migration.

Key words: *Muscardinus avellanarius*, microsatellites, population structure, barriers

Introduction

Most studies of hazel dormice (*Muscardinus avellanarius*) suggest that they do not cross open ground, including fields and roads. In 1991 Bright and Morris radio-tracked dormice in the woods of Southwest England and found that dormice would rather undertake a longer route than walk over open ground. Dormice are therefore considered to be weak in dispersal and even more so in open rural landscapes. Roads and larger open fields are regarded as insurmountable obstacles that could split and endanger a whole population (Richardson et al. 1997, Forman & Alexander 1998).

However, in recent years some contradictory information has been collected by various authors. Büchner (2008) found that hazel dormice in Saxony (Germany) occasionally overcome distances of even 250-500 m of open ground. A translocation study by Mortelliti et al. (2012) showed that dormice in Central Italy are able to cover distances of up to 106 m across open fields, whereas Chanin & Gubert (2012) described dormice occasionally crossing treeless- and shrubless gaps. Dormice were found in nest boxes installed on a central reservation of a motorway in Southwest England. Schulz et al. (2012) found dormice in roadside shrubs and on traffic islands in North and Central Germany, suggesting that dormice are not that troubled by the traffic. Ehlers (2012) even considered that species-rich roadside shrubs might function as potential dispersal corridors or habitats for hazel dormice. A recent non-genetic study by Kelm et al. (2015), conducted with a subset of our study animals, revealed road crossings by dormice using telemetry and capture-mark-recapture methods.

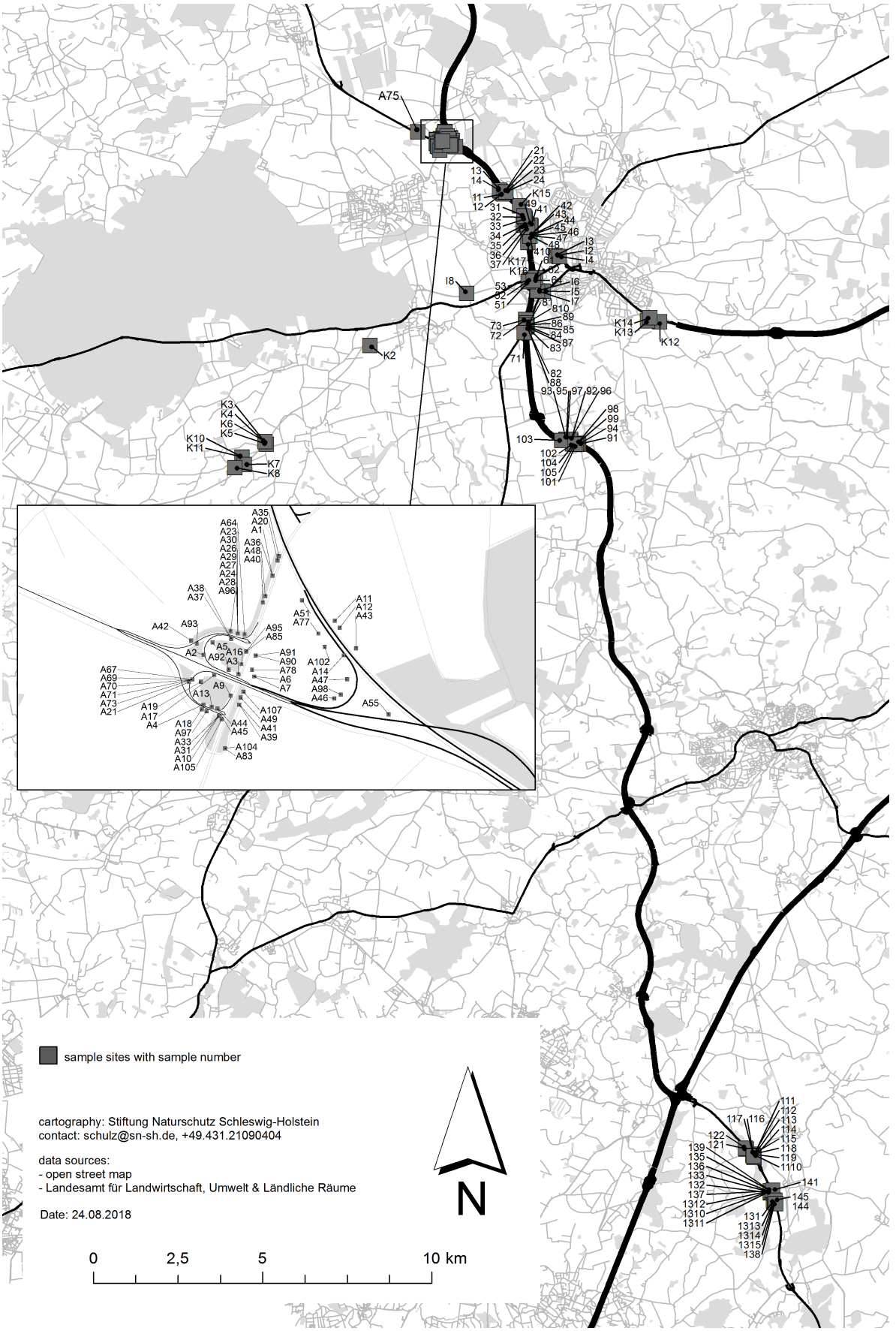

Fig. 1. Map of the study area along the motorway A21 in Northern Germany including samples sites and sample numbers.

These authors assumed that road crossings occurred frequently. Acquiring more knowledge about the impact of roads and roadside habitats on the life and population structure of hazel dormice is crucial for a realistic assessment of environmental factors governing the development of populations, as well as for effective action plans and roadside management (Büchner & Lang 2014).

Microsatellite analysis is a powerful tool for individual genetic profiling, for analysing small-scale population structure and for examining migration patterns over short geographic distances (Piertney et al. 1998, Gauffre et al. 2008, 2009). Genetic data on dormouse populations in Europe is available, but so far the respective studies were directed at assessing their phylogenetic history (Kramerov et al. 1999, Nunome et al. 2007, Mouton et al. 2012a, b) and levels of genetic variability (Naim et al. 2011a, b, Naim et al. 2014). The particular aspect of roads potentially acting as barriers to gene flow or, on the other hand, potentially facilitating dispersal has so far been largely neglected.

To elucidate the role of major roads in dormouse dispersal, an area in southeast Schleswig-Holstein (Northern Germany) was chosen comprising a 34 km section of the A21 motorway (in the last 5 km the road changes into the B404 federal highway), where dormice are found with a high consistency. Both, roads and roadside habitats, were built between the 1950s and 1970s and there are no cross-linking structures (fauna passages) to facilitate habitat connectivity over the motorway and highway respectively. Microsatellites were used to explore whether gene-flow between local population(s) west and east of the road takes place. Genetic diversity and genetic population structure within the study area were examined in order to detect past fluctuations in population size (bottlenecks) and to evaluate patterns of small-scale relationships among local populations of dormice.

Material and Methods

Sample collection and DNA extraction

A total of 195 tissue and hair samples were collected from hazel dormice along a 34 km section of the A21 motorway in southeast Schleswig-Holstein in northern Germany (Fig. 1). Samples were manually taken from animals inhabiting nest-tubes put out in roadside habitats, with the permission of the State Department of Agriculture, Environment and Rural Areas of Schleswig-Holstein (LLUR). Prior to microsatellite analysis the samples were stored at –20 °C and DNA was extracted by using the Invisorb® Spin Forensic Kit (Stratec Molecular) following the protocol for tissue and hair, respectively. In order to destroy PCR-inhibiting enzymes all samples were heated to 95 °C for 10 minutes.

Microsatellite genotyping

Ten microsatellites were used to assess genetic diversity: MavF10, MavG9, MavA5, MavF1-2, MavG6, MavB5, MavE3 (Naim et al. 2009), Mav21, Mav23, Mav28 (Mills et al. 2013). Polymerase chain reaction (PCR) amplifications were performed using the GoTaq Polymerase by Promega.

Amplification reactions contained 20-50 ng DNA, PCR Buffer, 1.5 mM Magnesium, 0.2 mM of each dNTP, 0.5 µM of each primer, 1.3 units Polymerase in 16 µl reaction volume in total. Foreward primers were labelled with either of the two fluorescent dyes Hex or Fam. Amplification using the thermo-cycler T Gradient PCR System 2800 by Biometra was carried out with an initial incubation at 95 °C for 5 min., followed by 40 cycles of denaturation at 94 °C for 1 min. 30 sec., primer annealing at primer specific temperatures (Naim et al. 2009, Mills et al. 2013) for 1 min. 15 sec. and extension of 72 °C for 1 min. 30 sec. and lastly a final extension of 72 °C for 10 min. Length of alleles was determined by the Institute of Clinical Molecular Biology in Kiel, using the capillary sequencer MegaBACE TM 1000 SEQ/GENO/SNP and data analysed using the software Peakscanner 1.0 (Applied Biosystems).

Test for errors

A test for null alleles and other typing errors was performed with the software Microchecker (van Oosterhout et al. 2004). In order to exclude identical samples (which could happen, when one individual is accidentally sampled twice) the test for identity was done with the software Cervus (Kalinowski et al. 2007). Samples were excluded, when identical at a minimum of nine out of ten microsatellite loci and double-sampling could not be ruled out on grounds of sampling locations, sex, age or sampling date. Thus 18 of 195 samples were excluded. The remaining 177 samples were included in further analysis. The data were also tested for linkage disequilibrium (LD) using the software Genepop (Raymond & Rousset 1995).

Population structure and spatial distribution

Population structure was analysed using the Bayesian clustering method, implemented in the programme Structure (Pritchard et al. 2000). To estimate the

number of subpopulations (k), ten independent runs of k from one to ten were performed with 500000 iterations and a burn in period of 50000. The admixture model was accepted, as individuals could originate from more than one population and allele frequencies were supposed to be not independent.

The highest logarithmic likelihood value (InP (D)) was elected and, in addition, the bar-plot diagrams were visually checked for the most probable number of groups. However, this can only be regarded as an indicator of the actual number of clusters (Pritchard et al. 2000).

Subpopulations assigned by the Bayesian clustering method were checked for differentiation between each other by using the weighed analysis (standard ANOVA) of genetic differentiation (Cockerham 1973) performed with Genepop. The level of separation was divided into three classes: separation (F_{ST} > 0.15, Frankham et al. 2010) medium separation (F_{ST} 0.14-0.10) and no separation (F_{ST} < 0.10, Chen et al. 2007). Furthermore, the pairwise genetic distances reported by Cavalli-Sforza & Edwards (1967) and by Nei (1978) were calculated using the software Genetix.

The detection of first generation immigrants was performed with the program Geneclass (Cornuet et al. 1999). The genetic distance-based method with the distance measured by Nei (1972), Cavalli-Sforza & Edwards (1967), Goldstein et al. (1995) and Paetkau et al. (2004) was used and the Monte-Carlo simulation algorithm was set at 10000 simulations with a threshold of scores at 0.01 (Paetkau et al. 2004). The probability that the individual was descended from the population in which it was found (geographically and by Bayesian clustering) was calculated.

For the additional landscape genetic calculation the Mantel test (Mantel 1967) and the software "Alleles in Space" (Miller 2005) were used for checking whether the genetic distance (Nei et al. 1983, Sumner et al. 2001) and the respective geographical distance among individuals were correlated. The test was conducted with 10000 replicates. A generalised Bonferroni-corrected spatial autocorrelation (Miller 2005) was also performed, giving an impression of the shape and pattern of the spatial relationship (Manel et al. 2003).

Genetic relationships

The genetic relationship between individuals was analysed using the software ML-Relate (Kalinowski et al. 2006). The program calculates the most likely relationship (e.g. parent-offspring, siblings etc.) between the two individuals (Blouin 2003, Wagner et al. 2006). The focus was set on individual pairs in the same population and therefore in direct geographical proximity. Only two subpopulations (Middle and South) were suitable for this analysis, as they were situated in immediate proximity to the road and sufficient individuals were sampled from both roadsides (Middle: n_{west} = 42, n_{east} = 24; South: n_{west} = 24, n_{east} = 5).

Genetic diversity estimates

Genetic variation within and among subpopulations was evaluated based on the polymorphism of the ten microsatellite loci. The number of alleles (A), allelic richness (A_R), and observed and expected average heterozygosity (H_o and H_e) for each of the identified subpopulations were calculated by using the software FSTAT (version 2.9.3.2). Tests for genotypic deviations from the Hardy-Weinberg equilibrium (HWE) (Guo & Thompson 1992) and calculation of the inbreeding coefficient F_{IS} (Wright 1922) were performed with Genepop. Relatedness of all individuals and within all subpopulations was calculated using the software Kingroup (Goodnight & Queller 1999) which calculates the mean relatedness (R) based on the maximum likelihood approach.

The software Bottleneck (Cornuet & Luikart 1997) was used in order to test for recent bottleneck events. Bottleneck detects departures from mutation drift equilibrium based on heterozygosity excess or deficiency, assuming two different allelic mutation models: stepwise mutation model (SMM, Cornuet & Luikart 1997) and two phase mutation model (TPM, DiRienzo et al. 1994). For TPM 95 % stepwise mutation model with 5 % multi-step mutations and a variance among multiple steps of 12 was assumed, as considered best for microsatellite data by Piry et al. (1999). Both the Wilcoxon signed-rank test and a sign test were used to determine the significance of a possible heterozygote excess under an equilibrium model. Bottleneck also describes the allele frequency distribution ("mode shift" indicator, Luikart & Cornuet 1998), which discriminates bottlenecked from stable populations.

Results

Testing errors

Linkage disequilibrium (LD) was found in 29 pairs of loci for all individuals pooled (p < 0.05). Separated into the five subpopulations, LD appeared in six pairs of loci for all specimens. LD of the individual subpopulations varied from zero to six and appeared in different pairings of loci. There were a minimum of nine families (31 individuals), each found together in

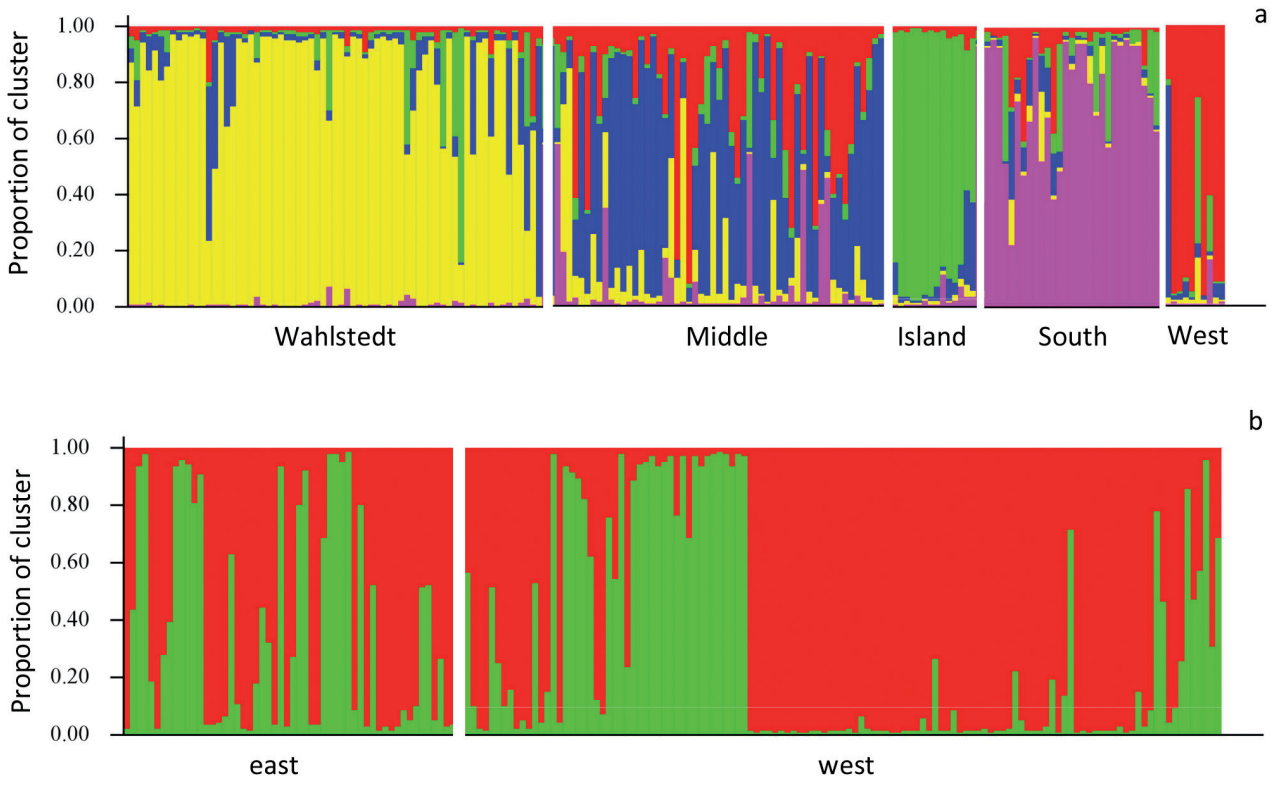

Fig. 2. Results of structure analysis for a) K = 5 assorted into the five subpopulations and b) K = 2 assorted into individuals east and west of the road. Assorted populations are separated by white columns.

one nest and therefore very likely to be closely related to one another. In order to test whether these closely related groups have an influence on the LD, one run was performed without the juvenile individuals. When only adult individuals were taken into account, the number of linked loci was zero. These results suggest that the LD pattern most probably results from a biased sample collection rather than from physical linkage between loci.

Population structure

Structure analysis (Fig. 2a) in combination with geographic distribution suggested the presence of five subpopulations: Wahlstedt (n = 68), Middle (n = 56), Island (n = 14), South (n = 29) and West (n = 10). Samples belonging to "Island" were collected within the geographic range of the Middle subpopulation and there was no obvious geographic reason for any separation.

Structure analysis (Fig. 2b) did not support the assumption of a genetic differentiation between individuals from the two roadsides (N_{west} = 123, N_{east} = 54). The F_{ST}-value amounted to 0.021, chord distance was 0.038, and genetic distance according to Nei (1978) was 0.022. Comparing only the two subpopulations closely adjacent to the road, Middle (located at the A21) and South (located at the B404), the F_{ST} values were 0.021 (chord distance: 0.088, Nei's distance: 0.037) for the Middle subpopulation and 0.020 (chord distance and Nei's distance: 0.057) for the South subpopulation.

F_{ST} values (Table 1) showed marked separation for five out of ten pairs of subpopulation and medium separation for three pairs. There was no separation between Wahlstedt-Middle and Middle-West. Although there was in fact no geographic difference between Island and Middle, F_{ST} yielded a medium value of 0.104. F_{ST} was highest between Wahlstedt and Island, which were approximately 4.6 km apart. Chord distances ranged from 0.063 between Wahlstedt and Middle to 0.227 between West and Island. All values except for the one for Wahlstedt and Middle were greater than 0.01. Genetic distances (Nei 1978) ranged from 0.102 between Wahlstedt and Middle to 0.370 between West and Island.

The Assignment test, based on four different genetic distance measures, revealed that the assignment is significant ($p \leq 0.05$). The run with the genetic distance by Nei (1972) resulted in five individuals and the genetic distance by Paetkau et al. (2004) resulted in yet another three individuals probably ($p \leq 0.01$) not descending from the assigned subpopulation. Of these

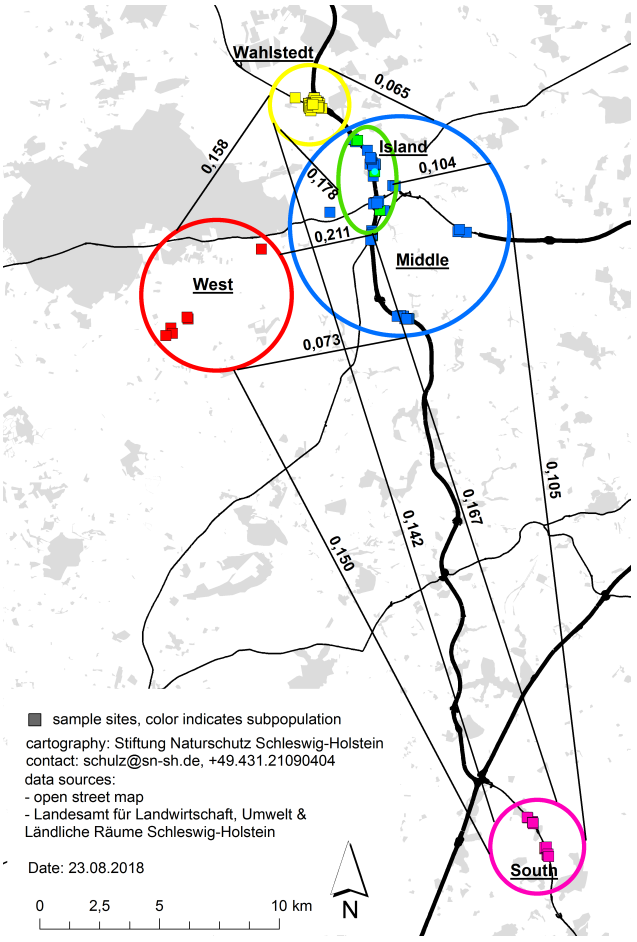

Fig. 3. Geographic distribution of all five subpopulations (coloured circles) with corresponding F_{ST} values.

eight individuals, four were assigned to the Middle, two to the South and one each to the Wahlstedt and the West subpopulation. The test with the two other distance measures (Cavalli-Sforza & Edwards 1967, Goldstein et al. 1995) revealed no incorrectly assigned individuals.

The Mantel test on correlation between genetic and geographic distance resulted in a slight positive correlation (r = 0.276). The investigation range extended over distances up to 35.6 km with a concentration on distances between zero and eight kilometers and a gap for distances between 16 and 22 kilometers. Spatial autocorrelation also suggested a slight increase in average genetic distance with increasing geographic distance. Genetic distance increased quite rapidly within distance classes of zero to three kilometers. With distances of three kilometres upward the increase became less apparent.

Genetic kinship

A total of 535 close kinships (coefficient of relationship 12.5 % or higher) between individuals was found (Table 2). In 217 (40.6 %) of these pairs the two individuals belonged to different road sides. The majority (198) of these pairs was found in the Middle subpopulation (A21) and only few such pairs (19) were found in the South subpopulation (B404). In the North a kinship relationship in 19.6 % of all pairings across the road was detected. In the South 15.8 % of the pairings were closely related to each other. In comparison the proportion of kinship pairs along the road was 14.7 % in the Middle and 22.3 % in the South.

Genetic diversity

The mean number of alleles per subpopulation ranged from 3.6 to 5.9 (Table 3). Calculated over all individuals, the mean number of alleles was 7.3. The South subpopulation exhibited a total of seven,

Table 1. F_{ST} values, chord distance (Cavalli-Sforza & Edwards 1967) and genetic distance (Nei 1978) – lower triangle – and approximate geographic distance of the population centers – upper triangle – between all pairs of subpopulations; **for F_{ST} values represents a strong separation, *represents a medium separation.

Subpopulation	Wahlstedt	Middle	Island	South	West
Wahlstedt	0	6.3 km	4.6 km	33.1 km	8.0 km
	0.064	0	(2.3 km)	25.6 km	
	0.063				
Middle	0.102				7.5 km
	0.178**	0.104*	0	27.5 km	
	0.148	0.126			
Island	0.102	0.166			7.6 km
	0.142*	0.105*	0.167**	0	
	0.173	0.130	0.185		
South	0.291	0.230	0.320		26.4 km
	0.158**	0.073	0.211**	0.150**	
	0.174	0.106	0.227	0.195	
West	0.302	0.143	0.370	0.361	0

Table 2. Observed number and portion of related pairs in the Middle and South subpopulation along and across the road. Portion refers to the all possible pairs in the respective group (along, across).

Population	Middle (N_{west} = 42, N_{east} = 24)			South (N_{west} = 24, N_{east} = 5)		
	total pairs	kinship pairs	portion	total pairs	kinship pairs	portion
along the road	1710	251	0.147	301	67	0.223
across the road	1008	198	0.196	120	19	0.158

Table 3. Genetic diversity estimates in subpopulations of dormice. n = sample size, A = mean number of alleles per locus, EA = number of exclusive alleles, A_R = allelic richness, H_e (H_o) = expected (observed) average heterozygosity, r = mean relatedness, F_{IS} = inbreeding coefficient, HWE = probability for deviation of genotypes from Hardy-Weinberg expectations, SD = standard deviation.

Subpopulation	n	A	EA	A_R	H_e	H_o	r	F_{IS}	HWE	SD
Wahlstedt	68	4.5	2	2.9	0.57	0.68	0.250	–0.219	0.782	0.007
Middle	56	5.9	5	3.8	0.62	0.57	0.049	–0.016	0.001	0.000
Island	14	3.7	0	3.1	0.49	0.42	0.231	0.100	0.001	0.000
South	29	5.2	7	3.9	0.65	0.54	0.120	0.063	0.000	0.000
West	10	3.6	1	3.3	0.53	0.54	0.185	–0.121	0.912	0.002
all	177	7.3		7.3	0.65	0.59	0.024		0.000	0.000

Table 4. Basic population genetic statistics per locus with average number of alleles, expected heterozygosity (H_e) and observed heterozygosity (H_o).

	F10	G9	A5	F12	G6	B5	E3	Mav 21	Mav 23	Mav 28	average
alleles (n)											
Wahlstedt	5	4	5	4	2	6	4	5	4	6	4.50
Middle	6	5	10	6	4	4	4	6	5	9	5.90
Island	3	3	6	3	3	5	3	3	5	3	3.70
South	5	6	7	4	3	5	5	6	5	6	5.20
West	4	4	5	3	2	1	3	4	4	6	3.60
all	10	7	10	8	5	6	5	6	6	10	7.30
expected heterozygosity (H_e)											
Wahlstedt	0.64	0.50	0.61	0.53	0.50	0.59	0.67	0.52	0.50	0.61	0.57
Middle	0.71	0.48	0.88	0.47	0.55	0.48	0.66	0.66	0.52	0.76	0.62
Island	0.31	0.29	0.57	0.09	0.54	0.63	0.60	0.59	0.64	0.61	0.49
South	0.65	0.77	0.78	0.54	0.44	0.53	0.70	0.70	0.66	0.77	0.65
West	0.51	0.61	0.72	0.51	0.46	0.00	0.48	0.71	0.63	0.70	0.53
all	0.69	0.63	0.82	0.53	0.53	0.54	0.73	0.68	0.56	0.76	0.65
observed heterozygosity (H_o)											
Wahlstedt	0.62	0.59	0.82	0.78	0.62	0.47	0.76	0.66	0.72	0.74	0.68
Middle	0.52	0.41	0.72	0.50	0.38	0.41	0.72	0.78	0.52	0.71	0.57
Island	0.33	0.33	0.33	0.08	0.17	0.75	0.33	0.67	0.25	1.00	0.42
South	0.66	0.69	0.45	0.62	0.48	0.45	0.55	0.72	0.38	0.38	0.54
West	0.30	0.70	0.70	0.60	0.30	0.00	0.50	0.90	0.60	0.80	0.54
all	0.55	0.54	0.69	0.60	0.47	0.44	0.67	0.72	0.56	0.69	0.59

the Middle five, Wahlstedt two and the Western subpopulation one exclusive allele. There was no exclusive allele in the Island subpopulation. Allelic richness ranged from 2.9 in Wahlstedt to 3.9 in the South subpopulation. The average allelic richness for all individuals was 7.3. H_e was 0.65 and H_o 0.59.

Mean relatedness between individuals in a subpopulation ranged from 0.049 in the Middle subpopulation and 0.250 in Wahlstedt. Relatedness over all individuals was 0.024. The data must be considered carefully, as sample size has a great influence on this measure and varied between

subpopulations. The inbreeding coefficient ranged from –0.219 for Wahlstedt to 0.100 for the Island subpopulation. Wahlstedt, Middle and West showed a slight heterozygote excess, indicated by the negative F_{IS} value. Only the subpopulations Wahlstedt and West were in a HWE, whereas Middle, Island, South and the total of all individuals showed significant deviations of genotypic proportions from Hardy-Weinberg expectations.

The bottleneck analysis for derivation from mutation-drift equilibrium revealed no significant heterozygote excess ($p < 0.05$) under any mutation model (data not shown). The allele frequency distribution showed a normal L-shape, which indicates that no recent bottleneck event has occurred in the population studied.

Discussion

Population genetic structure

The division of the 177 samples into five subpopulations by structure (Wahlstedt, Middle, West, South and Island) and corrected for geographically reasonable units is largely supported by the F-statistic. The populations did not seem to be completely separated, which is apparent from the cluster analysis. Some few individuals have a mixed profile or an assignment to a subpopulation which is geographically located some distance away from the location where they were found. Although the division is not complete, there seems to be some barrier between the locations of the five subpopulations.

F_{ST} values can be compared in general to the ones from a study by Kozakiewicz et al. (2009). Bank voles (*Myodes glareolus*) and yellow-necked mice (*Apodemus flavicollis*) living on two islands were investigated and compared to the mainland populations. Due to their greater mobility, yellow-necked mice were less influenced by isolation because of distance or water barriers (approximately 10 km from one shore to the other). The F_{ST} values between the populations of bank voles of islands and mainland varied between 0.2260 and 0.2673. There seemed to be very little genetic exchange and even hints of recent bottlenecks could be found in the island populations. The supposed barriers in the present study did not cause such a drastic genetic differentiation, but F_{ST} values were nevertheless higher than the ones Kozakiewicz et al. (2009) found for yellow-necked mice (island and mainland populations). A reason could be the lower mobility of dormice and maximum dormouse density in habitats.

The level of genetic variability ($H_e = 0.65$; $A_R = 7.3$) is within the range of the values of other European dormouse studies and other related species living in similar habitats within Europe, like yellow-necked mice and bank voles. Naim et al. (2011b) identified a somewhat higher value of $H_e = 0.70$ ($A_R = 8.62$) in one dormouse population in a study conducted in England. In another population, however, with 0.59 ($A_R = 6.16$) the value was somewhat lower. These authors were confident that these values are representative of a high genetic diversity. In another dormouse study by Mills et al. (2013) H_e was even lower (0.44). H_e in bank voles was slightly higher. It was 0.88 in France (Gauffre et al. 2008) and showed a mean of 0.82 in Poland (Gortat et al. 2015). Gortat et al. (2015) also found a mean H_e of 0.65 in yellow-necked mice living on an island in Poland. Four of the five subpopulations in the present study showed no reduction in variability. Only the island population had a H_e of 0.49 ($A_R = 3.1$), which is well below the average. This is probably because the sample consisted of only 14 individuals and some of them were closely related. It is noteworthy that the largest subpopulation Wahlstedt had the smallest allelic richness ($A_R = 2.9$) and a considerably higher observed heterozygosity than expected (Table 4). Heterozygote excess can be a hint of a recent loss of genetic variance and A_R is even known to be more sensitive to the loss of rare alleles than H_e (Greenbaum et al. 2014). A loss of alleles can indicate a recent gene drift. Wahlstedt seems to be densely populated (Kelm et al. 2015) and there is no obvious physical barrier which would discourage migration. Maybe the habitat is already occupied by enough dormice, so that new immigrants need to migrate further into other areas. Looking at the structure results (Fig. 2a), Wahlstedt is much more homogenous than for example the Middle subpopulation. Only a few immigrants can be detected and they seem not to have mixed with the original subpopulation and imported their genes. Looking at the relatedness (r), Wahlstedt and also Island consists of much more related individuals than Middle. But then Wahlstedt was a location with a dense collection of samples within a relatively narrow area, whereas the samples from Middle were taken here and there within an extensive area. A further monitoring of the genetic development in these subpopulations would be interesting to further examine the development of levels of genetic variability.

Genotype frequencies in the subpopulations Middle, Island and South deviated significantly from HWE. HWE is defined for an ideal population that is not influenced by evolution (Frankham et al. 2010). In reality there is no such population. Especially small subpopulations that do not or only rarely mix with other populations are likely to be in HW-disequilibrium. Interestingly, the Wahlstedt subpopulation which seems rarely to mix with the other subpopulations and

seems to be genetically homogenous, however, is in HWE. Still there can be other populations that are not included in this study, frequently interchanging with Wahlstedt. The fact that the subpopulation Middle, which is also represented by numerous samples, is not in HWE could be the result of continuous immigration and emigration. Newly imported genes would not have time to get established in the population. The relatively high allelic richness supports this scenario. The subpopulations Island, South and West are represented by too few samples to take the results for granted. The overall HW-disequilibrium can be explained by the differentiation among subpopulations. All in all there are 15 private alleles. If random mating between individuals from all subpopulations is prohibited or delimited, alleles will not freely distribute and therefore a HWE cannot be achieved. So the fact that there is no overall HWE, also corroborates our assumption of quite well defined subpopulations.

Inbreeding can be a result of small, isolated populations, as was shown by Zachos et al. (2007) for a red deer, *Cervus elaphus*, population in Schleswig-Holstein. The population of 50 individuals suffered from brachygnathy (shortened lower jaw) and low genetic variability. Also Epps et al. (2005) documented the consequence of inbreeding and showed a rapid reduction of genetic diversity. Their study animal, the bighorn sheep, *Ovis canadensis*, in a desert of California, was isolated by channels. The results of the present investigation suggest that neither bottlenecks nor inbreeding took place in the study area. Inbreeding coefficients were very small in all subpopulations. The highest value of 0.1 in the Island subpopulation is smaller than the average coefficient of one English population found by Naim et al. (2011b), which was 0.18. As Gaines et al. (1997) showed, some events are influencing nuclear DNA data late and only under particular circumstances. Due to effective population size being only one quarter of that of nuclear DNA, mtDNA might be more sensitive to genetic bottlenecks than nuclear DNA and sequencing studies are being performed to elucidate genetic relationships among our local dormouse subpopulations in more detail.

Significance of roads and roadside habitats

Table 1 shows, that the genetic distances between the subpopulations cannot be explained by the average spatial distance to the other subpopulations. The Island and West subpopulations are among the spatially closest and genetically most distant subpopulations, while the spatially most distant subpopulation South is genetically more related to the others.

By comparison the subpopulation West is genetically more differentiated from the examined populations than any other, even though there is little spatial distance and normal rural landscape between West and other populations. The main difference is, that West is the only population, which is not connected to the others by a well developed roadside shrub.

Subpopulation South is separated not only by a great distance (25-33 km), but also by the highly frequented motorway A1 which traverses the A21. Regardless of this remarkable distance and major roads, the F_{ST} value of 0.105 between West and Middle is comparatively low and indicates, that there is regular exchange between these populations over a long distance with ongoing gene-flow. Here a high-quality roadside habitat corridor could be enabling such a dispersal of populations, resulting in the colonisation of new habitats or at least the migration of single individuals resulting in low F_{ST} values even over long distances.

The genetic differentiation between Island and the other populations is difficult to explain. The spatial distance is low and there is no obvious physical barrier between these three subpopulations. Maybe the founders of the Island population recently immigrated into the area.

Our study site was included in the slightly larger-scale analysis of Mouton & Michaux (2013) and they found similar indications on population differentiation. Using eight microsatellites, samples of 49 individuals were studied. The result was a pattern of three clusters. One of them was the subpopulation Wahlstedt and the others were located northwest and east of Wahlstedt. The one east of Wahlstedt fits geographically to the Middle and Island subpopulation of this study. The one in the northwest is genetically related to individuals from South Denmark. Mouton and Michaux assume that the population differentiation arose from the phylogeographic background during historical recolonisation processes. Further population genetic investigations, with more samples as well as other approaches, are needed to explain why populations such as the Island exist.

In species as faithful to their habitat as the hazel dormouse, one would expect a high degree of isolation by distance (IBD). But in accordance with the results of Naim et al. (2011b) for a dormouse population in Southwest England, IBD was rather weak. A differentiation into subpopulations could not be explained by this weak IBD. Similar to the results of the present study, Naim et al. (2011b) found significant kinship coefficients over small distances, which can be expected due to the small-scale dispersal of families.

The rather slight increase of genetic distances with geographic distances (up to eight kilometres in the study by Naim et. al. 2014) indicates that gene flow occurs over great distances. According to our results, this holds true even for distances up to 34 kilometres. Thus, our data contradict the earlier assumption that dormice are sedentary. Another comparable study on bank voles in France (Gauffre et al. 2008), which took place in an area divided by a motorway, also showed a weak IBD and no dividing effect of the road. Their 500 km^2 study area was considerably larger than ours. Actually bank voles have a higher rate of reproduction, but with a home range of 0.05-0.3 ha (Radda 1968) a similar radius of action like dormice (Juškaitis 1997). Gauffre et al. (2008) ascribe the weak IBD to the high density and prevalence rate.

In accordance with other recent findings (Chanin & Gubert 2012, Schulz et al. 2012) a high abundance of dormice in the roadside greenery was found in this study. Based on the Mantel test (weak IBD) and looking at the genetic distances of the five subpopulations in this area (Fig. 3), it is obvious that the genetic distance along the road (Wahlstedt, Middle and South) is not related to the geographic distance. In contrast the genetic differentiation "overland" (West and Island) is comparatively high, even over short geographic distances. Further sampling may clarify this scenario. In the present study the two populations that were not in close proximity to the road had a very small sample size. The moderate genetic differentiation (F_{ST} values) and the close kinships across the road suggest that the roads themselves, A21 and B404 have little effect on the genetic structure of the dormouse population. Furthermore the very small difference in the proportion of kinship pairs along the roadside habitats and across the road (Table 2) could be seen as an even stronger reason to doubt that the road is a strong barrier to gene flow, when there is a well-developed habitat on either side of the road. The fact, that kinships of one generation (siblings and offspring) where found on different roadsides, indicates that also in the present active exchange takes place.

Comparing the locations at the A21 and the B404, which are different in level of use and road width, no significant difference in kinship relations and F_{ST} values could be found. Dormice crossed the road regardless of its type. However the sampling size east and west of the B404 was not even (24 to 5) and the total sampling size was much smaller than the one at the A21 (n = 78). So the data must be interpreted carefully and further research is necessary in order to substantiate this result and its implications.

Dormice benefit from the fact, that their nocturnal active phase coincides with the lowest traffic rates of the day. Traffic volumes as low as 34-39 motor vehicles per hour as a nightly average were counted in the study area (Kelm et al. 2015). This is very low compared to the daily traffic volume of 10000-30000 motor vehicles per day on the A21. Even though roads like the A21 are not necessarily barriers to gene flow, one cannot conclude that they have no negative effect at all for single hazel dormice, as it is not known how high mortality rates are among those that attempt to cross. Orlowski & Nowak (2006) found out that most of the mammal roadkill (approximately 40 %) were rodents. It is not impossible that single roadside habitats could act as a trap for individuals, by attracting them from surrounding land and then facing them with a high mortality risk on the road. That has yet to be proven. In our opinion, it is very unlikely that this could lead to a long-term and large-scale reduction of numbers of individuals. On the contrary, in our study site it is a proven fact, that roadside shrubs are a suitable habitat even for reproduction. Due to successful reproduction the population is viable, dispersal takes part and even significant barriers like motorways can be surmounted successfully and repeatedly. From this we assume that well developed and well managed roadside habitats can have a key role for dormouse conservation in fragmented landscapes, as they act as both a valuable "spring-board" for connections across roads and a corridor for connection along them. Removal of roadside habitats as a tool for the reduction of road mortality would most probably have negative effects on the local conservation status of the hazel dormouse and must be avoided unless there is strong evidence for a threat to the whole local population.

It would be interesting to know how the situation is on roads with more nocturnal traffic or with insurmountable central concrete walls, such as the motorway A7 north of Hamburg. One would expect a greater effect of the road on the dormouse populations there. Possibly the populations there are greatly affected by the busy traffic in combination with the concrete walls and safe crossing takes part at a much lower rate or is even impossible. Here we suggest a technical adaptation of the central concrete walls or the installation of insurmountable barriers between road and habitat to prevent hazel dormice from becoming roadkill. Destruction of roadside habitats is not an option as it will seriously affect local populations as gene-flow, both from across and along the road, would be reduced.

Acknowledgements

The authors wish to thank the Federal Agency for Nature Conservation for financing the project. We thank the Institute of Clinical Molecular Biology in Kiel for providing microsatellite analysis as supported in part by the DFG Cluster of Excellence "Inflammation at Interfaces" and "Future Ocean". We thank the technicians S. Greve, S. Arndt and T. Henke for technical support. Special thanks to Juliane Kelm and Annika Lange for their help and supply of samples during their fieldwork.

Literature

Blouin M.S. 2003: DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. *Trends Ecol. Evol. 18: 503–511.*

Büchner S. 2008: Dispersal of common dormice *Muscardinus avellanarius* in a habitat mosaic. *Acta Theriol. 53: 259–262.*

Büchner S. & Lang J. 2014: Die Haselmaus (*Muscardinus avellanarius*) in Deutschland-Lebensräume, Schutzmaßnahmen und Forschungsbedarf. *Säugetierkdl. Inform. 9: 367–377.*

Cavalli-Sforza L.L. & Edwards A.W.F. 1967: Phylogenetic analysis models and estimation procedures. *Am. J. Hum. Genet. 19: 233–257.*

Chanin P. & Gubert L. 2012: Common dormouse (*Muscardinus avellanarius*) movements in a landscape fragmented by roads. *Lutra 55: 3–15.*

Chen C., Durand E., Forbes F. & Francois O. 2007: Bayesian clustering algorithms ascertaining spatial population structure: a new computer program and a comparison study. *Mol. Ecol. Notes 7: 747–756.*

Cockerham C.C. 1973: Analyses of gene frequencies. *Genetics 74: 679–700.*

Cornuet J.M. & Luikart G. 1997: Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics 144: 2001–2014.*

Cornuet J.M., Piry S., Luikart G. et al. 1999: New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics 153: 1989–2000.*

DiRienzo A., Peterson A.C., Garza J.C. et al. 1994: Mutational processes of simple-sequence repeat loci in human populations. *Proc. Natl. Acad. Sci. U. S. A. 91: 3166–3170.*

Ehlers S. 2012: The importance of hedgerows for hazel dormice (*Muscardinus avellanarius*) in Northern Germany. *Peckiana 8: 41–47.*

Epps C.W., Palsbøll P.J., Wehausen J.D. et al. 2005: Highways block gene flow and cause a rapid decline in genetic diversity of desert bighorn sheep. *Ecol. Lett. 8: 1029–1038.*

Forman R.T.T. & Alexander L.E. 1998: Roads and their major ecological effects. *Annu. Rev. Ecol. Syst. 29: 207–231.*

Frankham R., Ballou J.D. & Briscoe D.A. 2010: Introduction to conservation genetics, 2nd ed. *Cambridge University Press, Cambridge, New York, U.K.*

Gaines M.S., Diffendorfer J.E., Tamarin R.H. & Whittam T.S. 1997: The effects of habitat fragmentation on the genetic structure of small mammal populations. *J. Hered. 88: 294–304.*

Gauffre B., Estoup A., Bretagnolle V. & Cosson J.F. 2008: Spatial genetic structure of a small rodent in a heterogeneous landscape. *Mol. Ecol. 17: 4619–4629.*

Gauffre B., Petit E., Brodier S. et al. 2009: Sex-biased dispersal patterns depend on the spatial scale in a social rodent. *Proc. R. Soc. Lond. B 276: 3487–3494.*

Goldstein D.B., Ruiz Linares A., Cavalli-Sforza L.L. & Feldman M.W. 1995: Genetic absolute dating based on microsatellites and the origin of modern humans. *Proc. Natl. Acad. Sci. U. S. A. 92: 6723–6727.*

Goodnight K.F. & Queller D.C. 1999: Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol. Ecol. 8: 1231–1234.*

Gortat T., Rutkowski R., Gryczynska A. et al. 2015: Genetic variability in island populations of two rodent species: bank vole (*Myodes glareolus*) and yellow-necked-mouse (*Apodemus flavicollis*). *Ann. Zool. Fenn. 52: 2–15.*

Greenbaum G., Templeton A.R., Zarmi Y. & Bar-David S. 2014: Allelic richness following population founding events – a stochastic modeling framework incorporating gene flow and genetic drift. *PLOS ONE 9: e115203.*

Guo S.W. & Thompson E.A. 1992: Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics 48: 361–372.*

Juškaitis R. 1997: Ranging and movement of the common dormouse *Muscardinus avellanarius* in Lithuania. *Acta Theriol. 42: 113–122.*

Kalinowski S.T., Taper M.L. & Marshall T.C. 2007: Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol. Ecol. 16: 1099–1106.*

Kalinowski S.T., Wagner A.P. & Taper M.L. 2006: ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Mol. Ecol. Notes 6: 576–579.*

Kelm J., Lange A., Schulz B. et al. 2015: How often does a strictly arboreal mammal voluntarily cross roads? New insights into the behaviour of the hazel dormouse in roadside habitats. *Folia Zool. 64: 342–348.*

Kozakiewicz M., Gortat T., Panagiotopoulou H. et al. 2009: The spatial genetic structure of bank vole (*Myodes glareolus*) and yellow-necked mouse (*Apodemus flavicollis*) populations: the effect of distance and habitat barriers. *Anim. Biol. 59: 169–187.*

Kramerov D., Vassetzky N. & Serdobova I. 1999: The evolutionary position of dormice (Gliridae) in Rodentia determined by a novel short retroposon. *Mol. Biol. Evol. 16: 715–717.*

Luikart G. & Cornuet J.-M. 1998: Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. *Conserv. Biol. 12: 228–237.*

Manel S., Schwartz M.K., Luikart G. & Taberlet P. 2003: Landscape genetics: combining landscape ecology and population genetics. *Trends Ecol. Evol. 18: 189–197.*

Mantel N. 1967: The detection of disease clustering and a generalized regression approach. *Cancer Res. 27: 209–220.*

Miller M.P. 2005: Alleles in space (AIS): computer software for the joint analysis of inter individual spatial and genetic information. *J. Hered. 96: 722–724.*

Mills C.A., Dawson D.A., Horsburgh G.J. et al. 2013: Isolation and characterisation of hazel dormouse (*Muscardinus avellanarius*) microsatellite loci. *Conserv. Genet. Resour. 5: 687–692.*

Mortelliti A., Santarelli L., Sozio G. et al. 2012: Long distance field crossings by hazel dormice (*Muscardinus avellanarius*) in fragmented landscapes. *Mamm. Biol. 78: 309–312.*

Mouton A., Grill A., Sarà M. et al. 2012a: Evidence of a complex phylogeographic structure in the common dormouse, *Muscardinus avellanarius* (Rodentia: Gliridae). *Biol. J. Linn. Soc. 105: 648–664.*

Mouton A., Grill A., Sarà M. et al. 2012b: Using phylogeography to promote dormouse conservation: the case of *Muscardinus avellanarius* (Rodentia, Gliridae). *Peckiana 8: 255–264.*

Mouton A. & Michaux J. 2013: Genetic analysis. In: Schulz B., Krog M., Reimers M. & Herty C. (eds.), Cross border conservation of the hazel dormouse. Presence, genetics, management and perspectives. *The Danish Nature Agency, Copenhagen*: *73–75.*

Naim D.M., Kemp S.J., Telfer S. & Watts P.C. 2009: Isolation and characterization of 10 microsatellite loci in the common dormouse *Muscardinus avellanarius*. *Mol. Ecol. Resour. 9: 1010–1012.*

Naim D.M., Telfer S., Sanderson S. et al. 2011a: Prevalence of multiple mating by female common dormice, *Muscardinus avellanarius*. *Conserv. Genet. 12: 971–979.*

Naim D.M., Telfer S., Tatman S. et al. 2011b: Patterns of genetic divergence among populations of the common dormouse, *Muscardinus avellanarius* in the UK. *Mol. Biol. Rep. 39: 1205–1215.*

Naim D.M., Telfer S., Tatman S. et al. 2014: Movement patterns and genetic diversity of wild and reintroduced common dormice, *Muscardinus avellanarius*. *Genet. Mol. Res. 13: 167–181.*

Nei M. 1972: Genetic distance between populations. *Am. Nat. 106: 283–292.*

Nei M. 1978: Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. U. S. A. 70: 3321–3323.*

Nei M., Tajima F. & Tateno Y. 1983: Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *J. Mol. Evol. 19: 153–170.*

Nunome M., Yasuda S.P., Sato J.J. et al. 2007: Phylogenetic relationships and divergence times among dormice (Rodentia, Gliridae) based on three nuclear genes. *Zool. Scr. 36: 537–546.*

Orłowski G. & Nowak L. 2006: Factors influencing mammal roadkills in the agricultural landscape of south-western Poland. *Pol. J. Ecol. 54: 283–294.*

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.*

Piertney S.B., MacColl A.D., Bacon P.J. & Dallas J.F. 1998: Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Mol. Ecol. 7: 1645–1654.*

Piry S., Luikart G. & Cornuet J.-M. 1999: BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered. 90: 502–503.*

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

Radda A. 1968: Populationsstudien an Rötelmäusen (*Clethrionomys glareolus* Schreber, 1780) durch Markierungsfang in Niederösterreich. *Oecologia 1: 219–235.*

Raymond M. & Rousset F. 1995: GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J. Hered. 86: 248–249.*

Richardson J.H., Shore R.F. & Treweek J.R. 1997: Are major roads a barrier to small mammals? *J. Zool. Lond. 243: 840–846.*

Schulz B., Ehlers S., Lang J. & Büchner S. 2012: Hazel dormice in roadside habitats. *Peckiana 8: 49–55.*

Sumner J., Rousset F., Estoup A. & Moritz C. 2001: "Neighbourhood" size, dispersal and density estimates in the prickly forest skink (*Gnypetoscincus queenslandiae*) using individual genetic and demographic methods. *Mol. Ecol. 10: 1917–1927.*

van Oosterhout C., Hutchinson W.F., Wills D.P.M. & Shipley P. 2004: Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes 4: 535–538.*

Wagner A.P., Creel S. & Kalinowski S.Z. 2006: Estimating relatedness and relationships using microsatellite loci with null alleles. *Heredity 97: 336–345.*

Wright S. 1922: Coefficients of inbreeding and relationship. *Am. Nat. 56: 330–338.*

Zachos F., Althoff C., von Steynitz Y. et al. 2007: Genetic analysis of an isolated red deer (*Cervus elaphus*) population showing signs of inbreeding depression. *Eur. J. Wildlife Res. 53: 61–67.*