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Original investigation

Rare migrants suffice to maintain high genetic diversity in an introduced island population of roe deer (*Capreolus capreolus*): Evidence from molecular data and simulations

Peter Steinbach[a,b], Mike Heddergott[a,*], Hannah Weigand[c], Alexander M. Weigand[c,a], Elodie Wilwert[a], Michael Stubbe[d], Bernd Helm[e], Robert E. Campbell[a], Annegret Stubbe[d], Alain C. Frantz[a]

[a] *Musée National d'Histoire Naturelle, 25 rue Muenster, L-2160 Luxembourg, Luxembourg*
[b] *University of Göttingen, Faculty of Chemistry, Tammannstraße 4, D-37077 Göttingen, Germany*
[c] *University of Duisburg-Essen, Faculty of Biology, Aquatic Ecosystem Research, Universitätsstr. 5, D-45141 Essen, Germany*
[d] *Martin-Luther University Halle-Wittenberg, Institute of Biology, Department of Zoology/Molecular Ecology, Hoher Weg 8, D-06099 Halle/Saale, Germany*
[e] *Landesforst Mecklenburg-Vorpommern, Forstamt Schuenhagen, Am Kronenwald 1, D-18469 Schuenhagen, Germany*

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ABSTRACT

As a popular game species, roe deer (*Capreolus capreolus*) have been subjected to a strong anthropogenic influence for centuries. While there have been attempts to introduce roe deer to some German islands, most of these initiatives were based on a small number of individuals and did not result in viable populations. The island population on Fehmarn became established, however, despite being founded by eight individuals with (supposedly) no subsequent restocking. Despite this strong founder effect, previous work has shown the contemporary population not to be genetically impoverished. The reasons for this were not entirely clear. Here, we use 13 microsatellite loci and population genetic techniques to test whether the high diversity resulted from a continuous genetic exchange with the mainland, whether a small number of immigrants maintained heterozygosity at a high level or whether the island was completely isolated and clandestine restocking by hunters another explanation for the observed high diversity. We confirmed that the genetic diversity on Fehmarn was high, but also show that the island population was genetically differentiated from the adjoining mainland. Results from different assignment methods identified a small number of mainland immigrants. Simulations provided support for the feasibility of a scenario of a small number of founders and limited natural immigration maintaining high genetic diversity despite population differentiation. Our simulation results also suggested that there will only be a relatively slight decrease in expected heterozygosity (H_e) over the next seventy generations and that there will be no need for restocking in the future.

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Introduction

The European roe deer (*Capreolus capreolus*) is the most common and widespread European ungulate (Mitchell-Jones et al., 1999). As a popular game species, it has been subjected to a strong anthropogenic influence for centuries. While the current annual hunting bag in Germany is just over one million individuals, roe deer almost disappeared from the country during the middle of the 19th century as a result of overexploitation (Arnold et al., 2015). While it has subsequently adapted to the cultural landscape, it can nevertheless be strongly affected by recent anthropogenic habitat fragmentation (Coulon et al., 2006; Holderegger and Di Giulio, 2010) and hunting activities (Kurt et al., 1993; Bonnot et al., 2013).

Similarly to other larger game species (Frantz et al., 2009, 2017), roe deer have been translocated in order to increase trophy quality, to restock populations and to introduce the species to new areas (Niethammer, 1963; Zachos et al., 2006b). In the early 20th century,

* Corresponding author.
E-mail addresses: mike-heddergott@web.de (M. Heddergott), hannah.weigand@uni-due.de (H. Weigand), alexander.weigand@uni-due.de (A.M. Weigand), elodie.wilwert@gmail.com (E. Wilwert), stubbe@zoologie.uni-halle.de (M. Stubbe), bernd.helm@lfoa-mv.de (B. Helm), campbell.earl.robert@gmail.com (R.E. Campbell), annegret.stubbe@zoologie.uni-halle.de (A. Stubbe), alain.frantz@mnhn.lu (A.C. Frantz).

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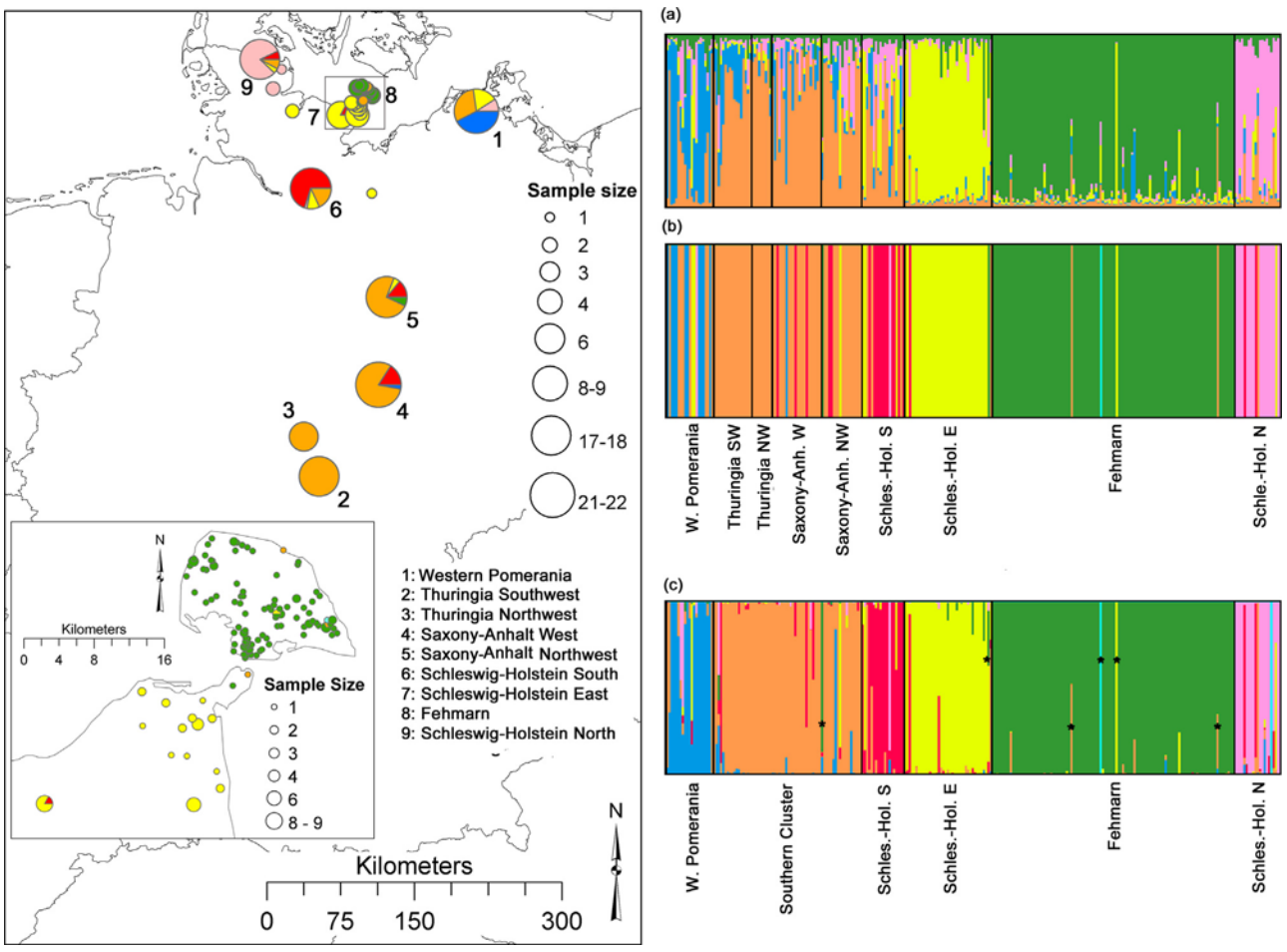

Fig. 1. Geographic distribution of the 273 roe deer samples and results of genetic clustering analyses. Left: Location of the nine pre-defined populations. Pie charts represent the average per cluster assignment values for all the individuals from a specific locality based on the individual-based clustering algorithm in BAPS ($K = 7$) and their size is indicative of the number of samples included. Different colours represent different genetic clusters and the colours correspond to the ones in the bar plots. Inset: Zoom in on Fehmarn and the adjoining mainland, location of inset given by square in main map. Right: Summary of the assignment analysis with (a) STRUCTURE ($K = 5$), (b) BAPS individual assignment ($K = 7$) and (c) GENECLASS (six predefined populations). Each individual is represented by a single vertical line, representing the individual's estimated proportion of membership to the different genetic clusters. Colours correspond to the clusters in the main figure. Based on the analysis of population genetic structure (see Results), we defined six genetic populations for the GENECLASS analysis. The two light-blue individuals in the GENECLASS bar plot could be excluded with $P < 0.01$ from all six pre-defined clusters. The asterisk indicates migrants from and to Fehmarn (or their descendants) that were identified by all three methods. W. Pomerania = Western Pomerania; Saxony-Anh. = Saxony-Anhalt; Schles.-Hol. = Schleswig-Holstein; W = West; SW = Southwest; NW = Northwest; S = South; E = East; N = North; Southern Cluster = Thuringia Southwest & Thuringia Northwest, Saxony-Anhalt West & Saxony-Anhalt Northwest.

there were attempts to introduce roe deer to some smaller German Baltic (Fehmarn) and North Sea islands (Norderney, Juist, Föhr; Vollmer et al., 1995; Niethammer, 1963). Most of these initiatives were based on a small number of founder individuals and did not result in viable populations. Small, isolated populations are indeed highly vulnerable to extinction as a result of demographic and environmental stochasticity. They are also likely to suffer from reduced genetic diversity and inbreeding depression (Frankham et al., 2010; Zachos et al., 2007). Viable island populations were therefore only obtained after multiple restocking (Föhr; Zachos et al., 2006b) or repeated introduction attempts (Fehmarn; Niethammer, 1963).

Animals were first introduced to Fehmarn (size: 187 km^2, Fig. 1) in the early 20th century. While around 50 deer were present before World War I, the population had disappeared by 1918. Again in 1935 three bucks and five does originating from Denmark (Zealand) were released on the island by local hunters (Niethammer, 1963). By 1955 the population had grown to an estimated 550 animals (Niethammer, 1963), while recent estimates arrive at a density of 6–7 individuals/km^2 (M. Lüthje, pers. comm.). According to hunters and local authorities, there have never been additional roe deer introductions to Fehmarn after 1935 (Zachos et al., 2006a).

Despite a severe genetic bottleneck at foundation and in contrast to other roe deer island populations (Thulin, 2006), Zachos et al. (2006a) showed that the roe deer population of Fehmarn was not genetically impoverished, but rather that it had diversity levels comparable to mainland populations. Also, the authors identified eight different mitochondrial control region haplotypes in 26 Fehmarn roe deer (while five does were originally released). The reasons for a high genetic diversity in a population that experienced a strong founder effect were not entirely clear. Due to lack of sampling on the nearby mainland (nearest population approx. 100 km from Fehmarn), it was not possible to infer whether the Fehmarn Sound – the narrow sea channel separating the island from the mainland with a minimum width of 600 m – represents a barrier to gene flow. There is a road-and-rail-carrying bridge over the Sound and roe deer are considered to be good swimmers (Danilkin, 1996). The high genetic diversity observed on Fehmarn might thus be explained by a continuous genetic exchange with the mainland and hence the absence of genetic structure. Alternatively, a small number of immigrants might be enough to maintain heterozygosity values at a high level (Keller et al., 2001). Roe deer have been reported, for example, to migrate to the island over the frozen

sea during harsh winters (Niethammer, 1963). Finally, the complete isolation of the island with clandestine restocking by hunters might be another explanation for the observed high diversity, as suggested by Zachos et al. (2006a)

The overall objective of the present research was to clarify the mechanism(s) driving the high diversity in the Fehmarn population. We analysed a microsatellite data set consisting of a larger number of samples from Fehmarn and the adjoining mainland, as well as from other German populations. We used different population genetic methods to assess the degree of isolation of the island population, to identify immigrants and emigrants and to compare genetic diversity levels on the island and the adjoining mainland. We then used simulations of population genetic parameters under different migration scenarios to provide independent support for our interpretations.

Material and methods

Sample collection

Between 2014 and 2016 we collected 108 roe deer muscle samples from Fehmarn, as well as a further 165 samples from a total of eight localities across northern Germany (Fig. 1; Table A1). These latter samples also contained 39 samples from the mainland immediately adjoining Fehmarn (referred to as Schleswig-Holstein East hereafter, Fig. 1). Tissue samples were stored in 96% absolute ethanol until extraction. Roe deer are a game species in Germany and can be harvested by licensed hunters outside the closed season without special permission. No animal was killed with the aim of providing samples for this study. All hunted individuals were legally shot and made available to the authors. Thirty-nine of the sampled animals were roadkill.

Laboratory work

DNA was extracted from tissue using an ammonium acetate-based salting-out procedure (Miller et al., 1988). We genotyped the roe deer samples using 13 microsatellite loci: BM1818, BM757, Cer14, CSSM003, CSSM016, ETH225, INRA11, OarCR26, OarFCB304, RM188, RT1, T156, T193 (Table A2). The loci were amplified in a total of five multiplex Polymerase Chain Reactions (PCRs), where each reaction contained 1 x QIAGEN Multiplex Master Mix (QIAGEN) and 0.2 or 0.6 μM of each primer (Table A2). The amplification reaction started with a 5-min denaturation at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing for 90 s (temperatures in Table A2) and extension at 72 °C for 90 s. The final incubation was at 68 °C for 10 min. PCR products were separated using an ABI 3730xl automated DNA sequencer (Applied Biosystems), and the data were analysed using GeneMapper version 4.0 (Applied Biosystems).

Statistical analysis

We tested for the significance of heterozygote deficiency or excess in the eight populations with ≥17 individuals (Table A1) using the Markov chain method in GENEPOP 3.4 (Raymond and Rousset, 1995), with 10 000 dememorisation steps, 500 batches and 10 000 subsequent iterations. The populations were tested for linkage disequilibria among loci using an exact test based on a Markov chain method as implemented in GENEPOP. The false discovery rate technique was used to eliminate false assignment of significance by chance (Verhoeven et al., 2005).

We used STRUCTURE v2.3.1 (Pritchard et al., 2000) to estimate the number of genetic sub-populations (K) present in the dataset. Ten independent runs for $K = 1–10$ were carried out with 10^6 Markov chain Monte Carlo (MCMC) iterations after a burn-in period of 10^5 iterations, using the model with correlated allele frequencies and assuming admixture. ALPHA, the Dirichlet parameter for the degree of admixture, was allowed to vary between populations. After deciding on the most probable number of subpopulations based on the log-likelihood values (and their convergence) associated with each K, we calculated each individual's percentage of membership (q), averaging q over ten runs. We also used BAPS v5.4 (Corander et al., 2004) to perform a population mixture analysis based on the clustering of individuals. This algorithm partitions the data into populations with non-identical allele frequencies. The program was run for $K = 2–10$ with 10 replications for each K.

We performed tests for isolation-by-distance (IBD) on the Fehmarn roe deer data set only by analysing genetic relatedness between pairs of individuals as a function of geographical distance, using the program SPAGEDI 1.5a (Hardy and Vekemans, 2002). The corresponding linear regression slope b offers a convenient measure of the degree of spatial genetic structuring (Hardy and Vekemans, 2002). As suggested by Vekemans and Hardy (2004), the kinship coefficient (F_{ij}; Loiselle et al., 1995) was chosen as a pairwise estimator of genetic relatedness, as it is a relatively unbiased estimator with low sampling variance. The slope was tested for a significant difference from zero by 10 000 permutations of locations of individuals (Hardy and Vekemans, 2002). We used GENETIX v.4.05 (Belkhir et al., 2004) to visualize the genetic distances between individual roe deer by means of a factorial correspondence analysis (FCA). The degree of genetic divergence between the different pre-defined populations was quantified using F_{ST} (Weir and Cockerham, 1984) in SPAGEDI and significant difference from zero was tested with 10 000 permutations of individual genotypes between populations.

GENECLASS 2.0.g (Piry et al., 2004) was used to calculate the probability of an animal belonging to a genetic population (exclusion probability) based on the Monte Carlo method of Paetkau et al. (2004). Based on the population genetic results, we defined six populations for GENECLASS analysis (see Results). We simulated 10 000 multi-locus genotypes and set the threshold for individual exclusion to 0.01. In wildlife forensics, a more stringent threshold for excluding animals from a population – such as $P < 0.001$–is considered necessary (Manel et al., 2002), but an exclusion threshold of $p < 0.01$ is normally used in ecological studies to identify genetic immigrants (e.g., Aspi et al., 2006; Clark et al., 2008; Frantz et al., 2017). Individuals were assigned to their most likely source population (assignment test) using the partial Bayesian approach of Rannala and Mountain (1997) implemented in GENECLASS. For each individual, we obtained assignment values for all six predefined populations and created bar-plots (as we also did for the STRUCTURE and BAPS assignment results) using the software DISTRUCT 1.1 (Rosenberg, 2004).

Expected heterozygosity values (H_e; Nei, 1978) were estimated for each of the six pre-defined GENECLASS populations using GENETIX 4.05.2 (Belkhir et al., 2004). Mean allelic richness per locus for each pre-defined European population was calculated with FSTAT v. 2.9.3 (Goudet, 1995) and measures standardised for a population size of 12 diploid individuals. Based on the per locus estimates, we used a Kruskal-Wallis test to test for difference in H_e and allelic richness.

Simulation study

The program quantiNEMO (Neuenschwander et al., 2008) was used to simulate the historical and future demography of the roe deer on Fehmarn in relation to its adjacent mainland population, and to infer changes in population genetic characteristics (i.e. H_e, F_{ST}). Rather than trying to formally re-create the empirical data set, we aimed to test the general feasibility of specific popula-

tion genetic scenarios only. The starting allele frequencies of the simulations were retrieved for all 13 loci from the empirical data set using Arlequin v3.5.2 (Excoffier and Lischer, 2010) and the ntrl_allelic_file option in quantiNemo. The starting allele frequencies for the simulated mainland population were calculated from the Schleswig-Holstein East pre-defined population (based on 39 individuals), whereas the Schleswig-Holstein North population (20 individuals) served as the source for the Fehmarn population. The latter was chosen due to its geographical proximity to the Danish founder population.

Simulations were performed for six different parameter settings, varying the carrying capacity of the island population (N_{max} = 500 or 1000) and disperal rate between both populations (m = 0.001, 0.0005, 0.0001). Both parameter spaces were chosen to best represent i) the historical and long-term average population sizes, respectively and ii) rare migration events (3, 1.5 and 0.3 migrants per generation). The census population size of the mainland population was kept constant (N_{max} = 3000). Ten replicates were run for each parameter setting for a total of 150 generations. After ten generations with maximum carrying capacity for both populations, eight individuals were randomly picked from the island population (N_{max} = 8) to simulate the bottleneck event which has occurred after translocation of founder individuals. The population size was allowed to recover after one generation of bottleneck (N_{max} = 500 or 1000 in generation 11). In the simulations, a fecundity rate of 2.5 was used to account for population growth, random mating was assumed and the population size was randomly down-regulated to the carrying capacity. Summary statistics, i.e. H_e of the island population and F_{ST} value between the island and the mainland population, were calculated for every generation and averaged over the 10 replicates.

Results

After correcting for multiple tests, four loci deviated from HWE in the Fehmarn population, but no more than one locus deviated in the remaining seven pre-defined populations (Table A3). Not considering Fehmarn, no locus deviated from HWE in more than two pre-defined populations after correcting for multiple tests (Table A3). No locus was excluded from further analysis. After correcting for multiple tests, we only observed one case of linkage disequilibrium in one predefined population (Fehmarn: *ETH225* & *BM757*; $P < 0.0008$).

The log-likelihood values of the STRUCTURE analysis gave highest support for the presence of five genetic clusters in the data set (Fig. A.1). Fehmarn formed a distinct genetic population, as did the animals sampled in the immediately adjoining area on the mainland (Schleswig-Holstein East; Fig. 1). The animals in the northern-most (Schleswig-Holstein North) and the north-eastern (Western Pomerania) population also formed a (mostly) distinct genetic unit. While the remaining five pre-defined populations formed a distinct population, they appeared to have been mostly admixed with the other clusters, with the degree of admixture apparently decreasing from north to south (Fig. 1). The individual-based BAPS clustering algorithm identified the likely presence of seven clusters (P = 0.968). In essence, this second algorithm identified Fehmarn, Schleswig-Holstein East, Schleswig-Holstein North, Western Pomerania as well as the four southern-most pre-defined populations as distinct clusters. The Schleswig-Holstein South pre-defined population, which was identified as highly admixed by STRUCTURE, formed a sixth cluster, while one cluster was formed by a single roe deer (S1072) sampled on Fehmarn. When only analysing data from Fehmarn, the STRUCTURE log-likelihood values did not provide evidence for genetic sub-structuring (Fig. A.2). The roe deer on Fehmarn were characterised by a significant, but relatively weak individual-based isolation-by-distance pattern (slope ± s.e. = −0.008 ± 0.002; $P < 0.001$).

An FCA showed the roe deer on Fehmarn as well as the Schleswig-Holstein East population to be distinct from the remaining reference populations (Fig. 2). The Fehmarn individual S1072 that formed a single-individual cluster in the BAPS analysis was also an outlier in the FCA analysis. F_{ST} values varied between 0.009 and 0.121, with the largest values observed in pairwise comparisons involving Fehmarn and Schleswig-Holstein East (Table 1). With one

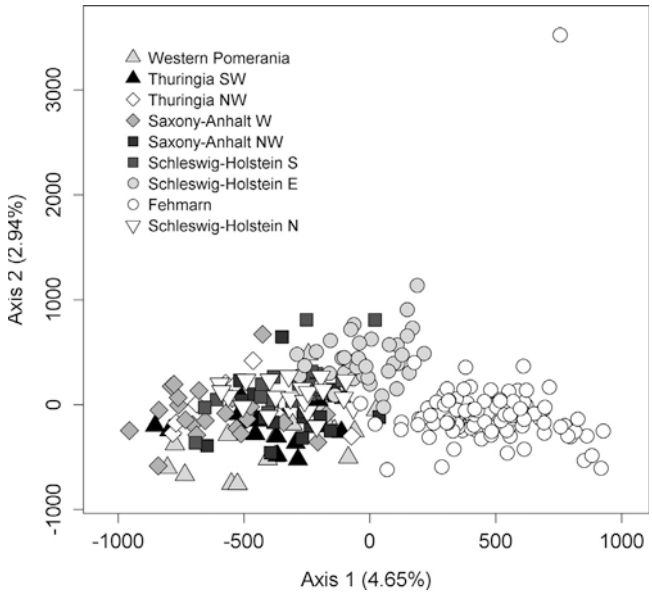

Fig. 2. Factorial correspondence analysis of German roe deer (n = 273). The analysis was based on 13 microsatellite loci. The percentage of the total variation explained by each of the two axes is given. The geographic location of the pre-defined populations is given in Fig. 1. The outlier in the upper right corresponds to individual S1072 (see Results).

Table 1
Genetic distance matrix of pairwise F_{ST} values of the nine pre-defined northern German roe deer populations. F_{ST} calculated according to Weir and Cockerham (1984) below the diagonal, significance values above. Non-significant values are in bold. W. Pomerania = Western Pomerania; Saxony-Anh. = Saxony-Anhalt; Schles.-Hol. = Schleswig-Holstein; W = West; SW = Southwest; NW = Northwest; S = South; E = East; N = North; Southern Cluster = Thuringia Southwest & Thuringia Northwest, Saxony-Anhalt West & Saxony-Anhalt Northwest.

	W. Pomerania	Thuringia SW	Thuringia NW	Saxony-Anh. W	Saxony-Anh. NW	Schles.-Hol. S	Schles.-Hol. E	Fehmarn	Schles.-Hol. N
W. Pomerania		0.002	0.002	0.000	0.000	<0.001	<0.001	<0.001	<0.001
Thuringia SW	0.037		0.104	**0.203**	0.004	<0.001	<0.001	<0.001	<0.001
Thuringia NW	0.073	**0.021**		**0.154**	**0.118**	<0.001	<0.001	<0.001	<0.001
Saxony-Anh. W	0.042	**0.009**	**0.015**		**0.060**	<0.001	<0.001	<0.001	<0.001
Saxony-Anh. NW	0.042	0.026	**0.018**	**0.013**		<0.001	<0.001	<0.001	<0.001
Schles.-Hol. S	0.063	0.064	0.070	0.043	0.054		<0.001	<0.001	<0.001
Schles.-Hol. E	0.129	0.096	0.086	0.108	0.083	0.088		<0.001	<0.001
Fehmarn	0.121	0.086	0.103	0.100	0.073	0.121	0.095		<0.001
Schles.-Hol. N	0.081	0.066	0.081	0.058	0.053	0.075	0.076	0.088	

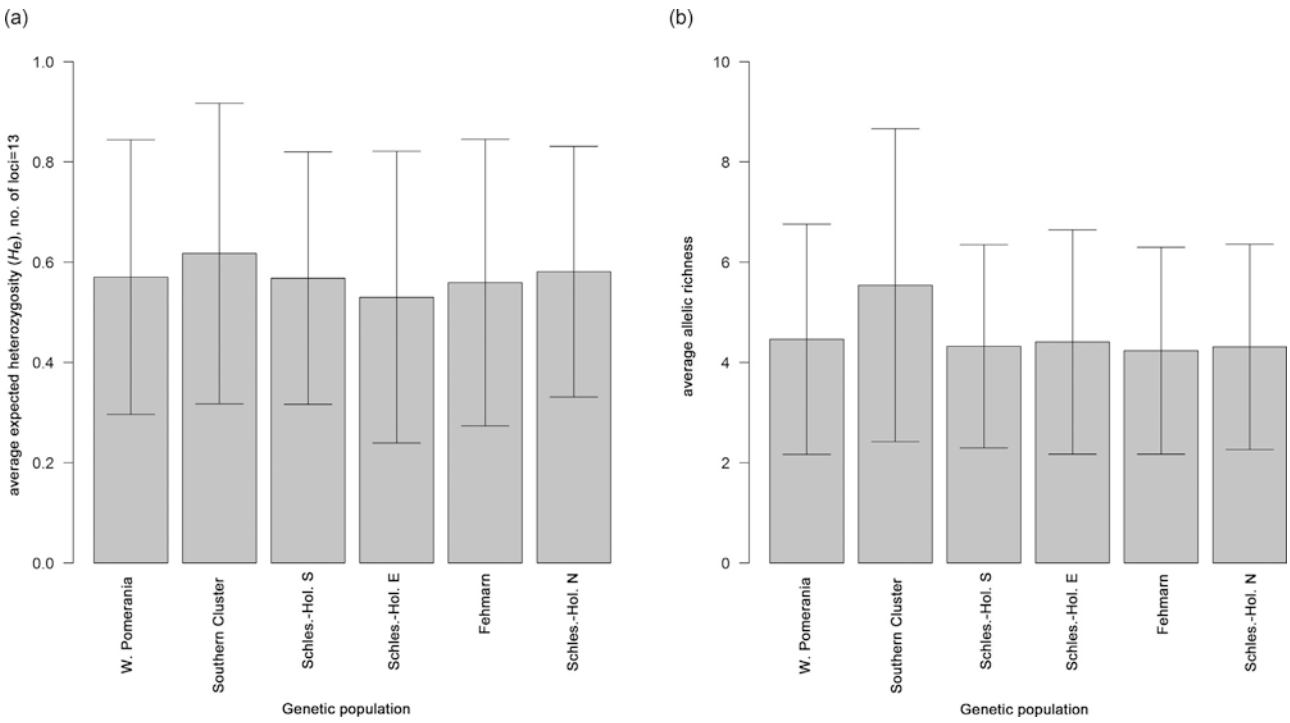

Fig. 3. Summary of genetic diversity statistics obtained for the six genetic populations defined in this study. Results for (a) average expected heterozygosity (Nei, 1978) and (b) average allelic richness based on a standardised population size of 12 individuals. W. Pomerania = Western Pomerania; Schles.-Hol. = Schleswig-Holstein; S = South; E = East; N = North; Southern Cluster = Thuringia Southwest & Thuringia Northwest, Saxony-Anhalt West & Saxony-Anhalt Northwest.

exception, pairwise F_{ST} comparisons between the four southernmost populations (Thuringia Southwest & Thuringia Northwest, Saxony-Anhalt West & Saxony-Anhalt Northwest) were not significant.

Considering all results of the analysis of the population genetic structure, we used six predefined populations for the subsequent analyses (GENECLASS, genetic diversity): Western Pomerania, the four southern populations (the Southern cluster), Schleswig-Holstein South, Schleswig-Holstein East, Fehmarn and Schleswig-Holstein North. Two animals could be excluded with GENECLASS at the $P < 0.01$-level from all six pre-defined populations: animal S1072 from Fehmarn again as well as one animal from Schleswig-Holstein North (Fig. 1). All other animals were assigned with $P \geq 0.05$ to one of the six predefined populations. Altogether four Fehmarn individuals (including S1072) were identified by all three assignment methods (STRUCUTRE, BAPS, GENECLASS) as either being non-native to the island, or as having an admixed ancestry (Fig. 1). The STRUCTURE results suggest that one of these Fehmarn animals was a recent migrant, as it was assigned with $q = 0.94$ to the adjoining mainland population. Similarly, two deer that were native to Fehmarn, or that had ancestors that were native to Fehmarn, were identified on the mainland (Fig. 1).

Average H_e values varied between 0.530 (Schleswig-Holstein East) and 0.617 (Southern cluster; Fig. 3). The observed heterozygosity values were very similar to the H_e values and varied between 0.503 (Fehmarn) and 0.602 (Schleswig-Holstein North; Fig. A.3). The largest differences between H_e and H_o were observed on Fehmarn and in the South Cluster (Fig. A.3). More loci deviated from HWE in these two populations than in the remaining four (Table A.3). Average allelic richness varied between 4.23 (Fehmarn) and 5.54 (Southern cluster; Fig. 3). While average allelic richness was lowest for Fehmarn, all clusters but the Southern cluster had very similar estimates (Fig. 3). There was no significant difference in H_e ($H = 2.706$, $d.f. = 5$, $P = 0.745$) and allelic richness ($H = 2.087$, $d.f. = 5$, $P = 0.837$) between the six different genetic clusters.

The simulated translocation and bottleneck event ($t = 10$ generations, $N_{max} = 8$, Fig. 4) reduced the census population of the island population to eight individuals, recovering after approx. 20 generations ($t = 30$ generations) to the maximum simulated carrying capacity ($N_{max} = 500$ and 1000). The bottleneck led to a sudden drop of H_e (Fig. 4), whose effect was prominent for the first ten generations. In contrast to the strong reduction of H_e values when migration was lowest, a migration rate of 0.05–0.1% per generation (i.e. 1.5–3 mainland individuals) resulted in relatively stable, high values of H_e (0.50–0.54), even after 140 simulated generations. The simulated genetic differentiation (F_{ST}) between the island and the mainland population increased with decreasing migration rates (Fig. 4). A migration rate of 0.01% led to an initial rise in population differentiation after the bottleneck, with subsequent high and relatively stable F_{ST} values (0.16–0.19). Higher migration rates led to lower, but relatively stable levels of genetic differentiation ($F_{ST} = 0.07$–0.08 for 0.05%; $F_{ST} = 0.05 - 0.06$ for 0.1%). The census population size of the island population only showed a minor effect on H_e and F_{ST}.

Discussion

We analysed the population genetic structure and genetic diversity of the roe deer populations using 13 microsatellite loci. A larger number of loci deviated from HWE in the Fehmarn population, but rather than being due to an intrinsic problem with the loci, this was in all likelihood due to population genetic processes such as the isolation-by-distance pattern (Frantz et al., 2009), the presence of immigrants or closely-related individuals in the data set (Anderson and Dunham, 2008).

The roe deer is a philopatric species that is characterised by short dispersal distances of normally less than 5 km (Stubbe, 1990; Coulon et al., 2006). It can also be strongly influenced by recent anthropogenic habitat fragmentation (Coulon et al., 2006; Breyne et al., 2014) and particularly by the presence of motorways (Kuehn

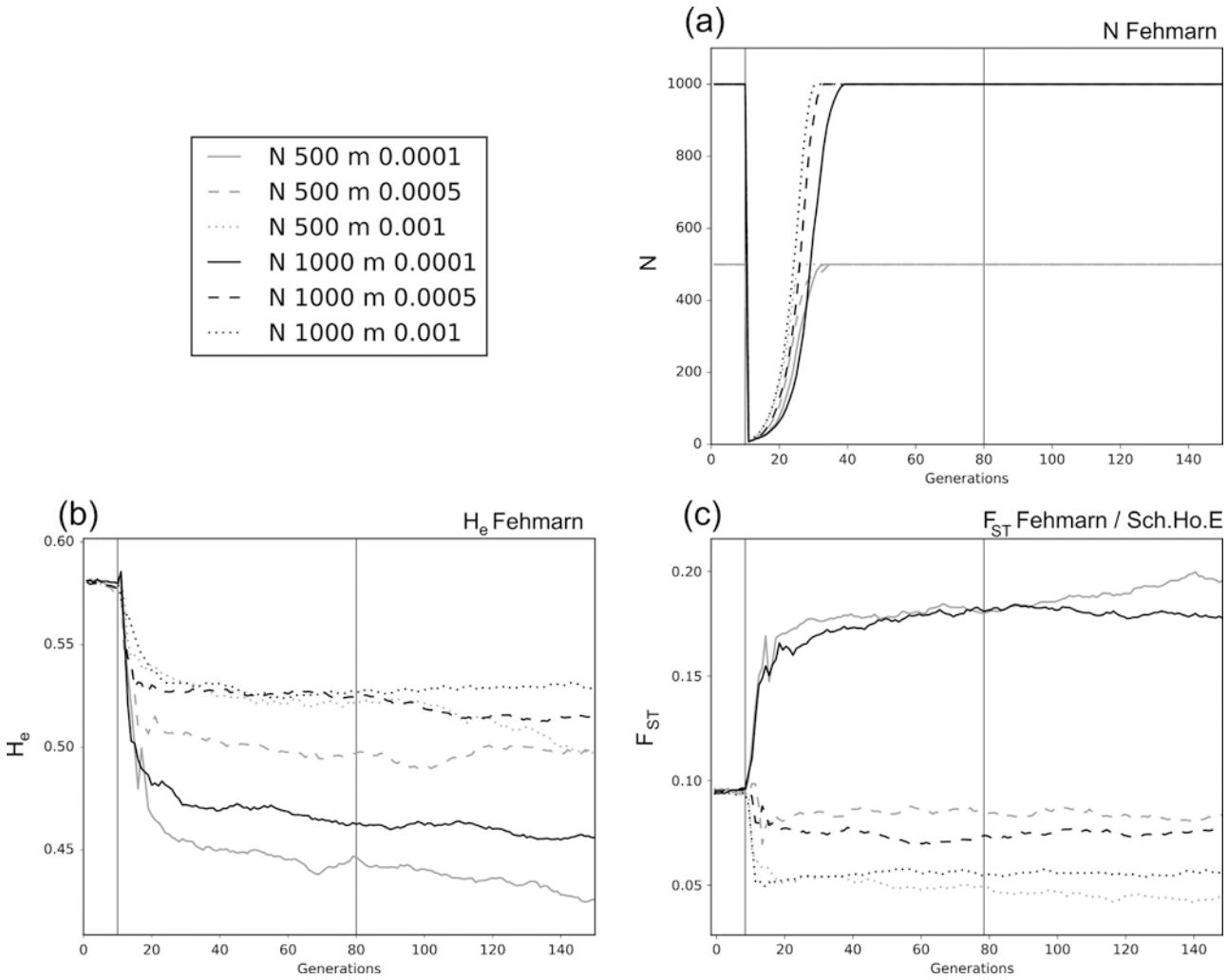

Fig. 4. Summary statistics for the simulation study under six parameter settings of carrying capacity (N_{max}) and number of migrants per generation (m). (a) Consensus population size for the simulated island population, including the simulated bottleneck event after 10 generations. (b) Expected heterozygosity (H_e) for the island population. (c) Genetic differentiation (F_{ST}) between the simulated mainland and island populations.

et al., 2007; Holderegger and Di Giulio, 2010; Hepenstrick et al., 2012). Hence, a pattern of small-scale genetic differentiation might be expected. While each pre-defined population in the northern study area indeed formed a distinct cluster, the four southern sampling areas where all part of one genetic partition (based on cluster analyses and F_{ST} values), despite being distributed over a similar geographic area. This pattern might be explained by Schleswig-Holstein being highly fragmented with few forested areas, while Thuringia and Saxony-Anhalt have larger forested areas (Borkenhagen, 2014; Polley et al., 2016), enabling population connectivity. Despite short dispersal distances being the norm in roe deer, most clusters contained admixed individuals or immigrants. On the one hand, this could be taken as confirmation that roe deer disperse further than inferred by traditional field methods based on the recapture of marked individuals (Koenig et al., 1996). On the other hand, the number of microsatellite loci employed here was relatively small and it is likely that the results of the assignment tests would be clearer when using a larger number of loci.

The highest genetic diversity, both in terms of H_e and allelic richness, was obtained in the Southern genetic cluster, indicating reduced effects of genetic drift due to larger effective population sizes or higher population connectivity compared to the northern populations. However, the pattern in genetic diversity could also be explained by a general south to north gradient in genetic diversity (Hewitt, 1999; Frantz et al., 2014). Results by Wang and Schreiber (2001) suggest that isolation and habitat fragmentation are better at explaining the genetic diversity at the national scale than geographic location. The observation of higher genetic diversity in the south might also be coincidental due to the small number of pre-defined populations analysed here. A detailed large-scale study using a large sample size and a larger number of microsatellite loci would clearly be of interest to analyse roe deer dispersal and the factors driving population connectivity or the lack thereof (see also Wang and Schreiber, 2001).

All our population genetic results agree that the roe deer population on Fehmarn is clearly differentiated from the population on the adjoining mainland as well as from other populations. Despite roe deer being good swimmers (Danilkin, 1996), the Fehmarn Sound clearly represented a significant barrier to gene flow. It is therefore perhaps not surprising that the deer had been absent from the island (given the small number of island immigrants per generation) and that the first attempt at introduction in the early 20th century was unsuccessful (given the demographic fluctuations associated with founder events). Similarly, the North Sea islands where roe deer introductions have been unsuccessful are even fur-

ther from the mainland and the populations may have significantly suffered from the lack of genetic exchange.

On the other hand, results from the different assignment methods showed that the strait is not an impermeable barrier. The STRUCTURE results suggested that at least one Fehmarn animal was a recent immigrant. One further island animal could be excluded by GENECLASS from all six reference clusters and therefore immigrated from a non-sampled, but genetically differentiated population. While it is theoretically possible that hunters had released this individual, it appears unlikely given the high roe deer density currently observed on the island. Whereas at least two further animals appeared to have admixed island/immigrant ancestry, their exact number is difficult to ascertain since the performance of different assignment methods varies depending on the degree of population differentiation and the number of loci used (Manel et al., 2002).

In spite of having marginally the lowest allelic richness of the six genetic clusters, our results essentially confirm the conclusion by Zachos et al. (2006a) that the genetic diversity of the roe deer population on Fehmarn is comparable to the mainland populations. It seems *a priori* perhaps surprising that a genetically differentiated island population, that was founded by eight individuals more than eighty years ago, exhibits a high level of genetic diversity. However, we found evidence for low background levels of immigration. Keller et al. (2001) genetically analysed an island population of a song sparrow (*Melospiza melodia*) that underwent a severe population reduction (95 % mortality). Despite the birds' shorter generation time, our results are in line with those of this natural experiment. Despite crashing to eight breeding birds, the population regained pre-bottleneck genetic diversity levels within a few years due to similarly low levels of immigration (1–3 animals/year) as observed in our study.

We performed simulations to further evaluate the possibility that a small number of founders and limited natural immigration could lead to the observed high genetic diversity and population differentiation. While many scenarios could be simulated, we focussed on a limited range of parameters chosen to best represent the actual demographic history of our study populations. We did not have information on the allelic diversity of the Danish founder individuals, but took allele frequencies from our northernmost pre-defined population as a proxy for the genetic make-up of the founder population. Roe deer are common on Zealand (Baagøe and Secher Jensen, 2016) and the exact geographic origin of the founder animals is not known. Furthermore, it is impossible to know how stable allele frequencies of the founder population have been over the last eighty years. It is therefore clear that we did not test the possibility of formally re-creating the empirical data set, but that we aimed only to test the general feasibility of a specific population genetic scenario.

Our simulations confirmed that after the initial introduction, no further human intervention would be necessary to explain the observed population structure and genetic diversity. Irrespective of population sizes and number of migrants, but assuming a fecundity rate of 2.5, the original population size (i.e. 500 or 1000) was reached in the simulated island population after twenty generations, fitting well with the observation that twenty years after the translocation, the population size had risen to an estimated 550 individuals. Both the results simulating 1.5 and 3 migrants from the mainland per generation show that, after an initial bottleneck-induced drop, heterozygosities can be maintained at relatively high levels, while fewer migrants lead to a more important drop in H_e. The best fit between the observed and expected level of genetic differentiation was obtained when simulating 1.5 migrants per generation, with more or fewer migrants leading to lower or substantially higher F_{ST} values, respectively. In summary, our simulations suggest that 1.5 mainland migrants per generation permits the maintenance of high genetic diversity and of the differentiation levels observed empirically.

In conclusion, the population genetic data and the simulations lend strong support to a scenario of no human intervention after the initial introduction, with one to three roe deer per generation immigrating to the island from the nearby mainland. While roe deer may walk over the frozen sound during harsh winters (Niethammer, 1963), the freezing-over of the sound is a fairly rare event. They are more likely to immigrate to the island by swimming across the 600 m that separate the mainland from the island at the sound's narrowest point. Also, we cannot categorically exclude the possibility that roe deer walk across the road-and-rail-carrying, 963-m-long Fehmarn Sound Bridge. While there are indications that roe deer have immigrated to the island before 1935 (Niethammer, 1963), they clearly did not manage to establish a viable population.

Although our study does not indicate human intervention after the initial introduction, it is impossible to state with certainty that there have been no additional releases. While five does were initially introduced to the island, Zachos et al. (2006a) observed eight different mitochondrial control region haplotypes in 26 Fehmarn roe deer. Yet, only six were private but two shared with the investigated mainland populations (with one being very widespread). It is of course impossible to know whether the additional haplotypes originated from repeated human-mediated introduction in the years after 1935, as suggested by Zachos et al. (2006a), or from natural immigration to the island. Given the high roe deer density currently observed on the island, it is unlikely that roe deer have been released by hunters recently.

While translocations of ungulates throughout Europe have been common for centuries (Niethammer, 1963; Apollonio et al., 2014), they blur natural structures and risk the introduction of pathogens into potentially immunologically naïve populations (Frantz et al., 2017). Clandestine translocations are therefore, generally, not to be recommended and are illegal in a number of countries (e.g. Frantz et al., 2009, 2017). Our simulation results suggest that, if the demographic parameters remain constant, there will only be a relatively slight decrease in expected heterozygosity over the next seventy generations for roe deer on Fehmarn. Whether or not humans have repeatedly introduced roe deer to Fehmarn in the past, it seems unlikely that restocking will be necessary in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.mambio.2017.11.009.

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