Genetic structure of badger populations in a fragmented landscape: how do barriers affect populations on a genetic level?

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Abstract: The expansion of urban area, accompanied by the development of infrastructure, leads to more fragmented natural living space and isolation of wild populations of plant and animal species, including the badger (Meles meles). Fragmentation enhances the influence of genetic drift in these isolated populations. In order to reconnect fragmented populations, corridors have been implemented as mitigation measures. It is unclear, however, whether these measures fully mitigate the effects of fragmentation. The aim of this study was to measure genetic differentiation between fragmented populations of badgers by establishing the population structure of badgers in a fragmented area and examining the influence of barriers on genetic differentiation. This study was carried out in the Utrechtse Heuvelrug, Province of Utrecht, the Netherlands. Tissue samples were collected from road killed and trapped badgers, and faecal samples were collected for a selected group of clans. Microsatellites were used to study population structure of badgers. Population structure of badgers on either side of a major road, the A27 motorway, differed substantially. The degree of heterozygosity between both populations appeared to be similar, allelic diversity however was much lower for the population west of the A27 motorway. This barrier may therefore limit gene flow despite implementation of multiple corridors. Long–term studies may reveal to what extent corridors mitigate the barrier effect by major roads.

Keywords: badger, Meles meles, population structure, fragmentation, barrier, corridor, gene flow.

Introduction

Terrestrial species continue to live under pressure for living space, whilst land use changes due to human population growth and land becomes fragmented as roads cut through natural landscapes (Meyer & Turner 1992). Fragmentation also leads to reduction of suitable natural living space. Habitat loss has large, consistently negative effects on biodiversity (Fahrig 2003). Fragmentation of habitat amplifies the negative impact on biodiversity as it sets off time delayed biodiversity loss (Krauss et al. 2010). Species on high tropic levels have smaller population sizes and greater mobility and are, therefore, more susceptible to habitat fragmentation (Gilbert et

al. 1998). By affecting different trophic levels, fragmentation may disrupt whole communities and ecosystems might collapse. Furthermore, fragmentation exacerbates edge effects and negatively affects population size of many species (Debinski & Holt 2000, van Strien et al. 2007).

The indirect effects of fragmentation might be underestimated, since populations may become isolated from each other as infrastructure and urban development limit migration chances, making populations vulnerable to the effects of inbreeding and genetic drift. The influence of habitat fragmentation on genetic variability of meta-populations has therefore been of recent scientific interest. Significant differences in genetic differentiation and in allelic diversity as a result of inbreeding were found in fragmentened populations of flightless ground beetle (Carabus violaceus), European tree frog (Hyla arborea), dice snake (Natrix tesselata), and desert bighorn sheep (Ovis canadensis nelsoni) and have led to genetically distinct populations in, for instance, the Florida black bear (Ursus americanus floridanus) (Gautschi et al. 2002, Keller & Largiader 2003, Andersen et al. 2004, Epps et al. 2005, Dixon et al. 2007). As fragmentation can lead to isolation, gene flow may become limited and the chance of genetic drift increased.

In order to reconnect fragmented populations, corridors have been implemented as mitigation measures. It is unclear however whether these measures fully mitigate the effect of fragmentation. Multiple methods have been applied to determine which species make use of corridors (van der Grift 2010). So far, most studies have demonstrated that corridors are frequently used to cross barriers throughout the year, during day and night, by multiple species and thus mitigate fragmentation effects as they provide safe passage and significantly reduce road mortality (Veenbaas et al. 2003, van der Grift 2005, Dekker & Bekker 2010, Rytwinski et al. 2016). Corridors prove to be especially effective for mustelids, for instance, by increasing individual life expectancy (Vink et al. 2008, van den Akker 2014). Most methods used in studies on reconnecting populations through corridors, however, lack the ability to identify specific individuals, and with that: actual dispersal or migration and gene flow facilitated by corridors. Sawaya et al. (2014) found bidirectional gene flow and genetic admixture in grizzly bears (Ursus arctos) but did not find this in black bears (Ursus americanus) within the same, with corridors reconnected, fragmented study area. Corridors can thus be useful to reconnect populations but their effectiveness to restore or maintain gene flow may depend on the species. Since corridors are intended to facilitate several species, it is difficult to determine whether the use of corridors is a fully effective measure to mitigate the effect of fragmentation.

We aimed to establish the population structure of badgers (Meles meles) in a fragmented area and to examine the influence of barriers on genetic differentiation. Dispersal of badgers is often to one of the neighbouring clans and rarely takes place over longer distances (Christian 1994, Rogers et al. 1998). Settling into another clan is a gradual process that may take up to nine months (Roper et al. 2003). The dispersal strategy of badgers, where clans act as stepping-stones for gene flow, makes the badger a suitable species to study genetic differentiation. Since roads function as an obstacle and as such territory bounderies, roads might directly affect gene flow. Corridors may mitigate this effect as they facilitate safe passage (Gilbert et al. 1998).

The study was conducted in a heavily fragmented area: the Utrechtse Heuvelrug. Motorways around Utrecht were found to function as territoria boundaries for badgers, adding weight to the assertion that elements such as motorways act as barriers and fragments habitat (Mulder 2016). The badger population in Utrecht originates from a small population located at Einde Gooi in the 1980s but has since spread across the Utrechtse Heuvelrug

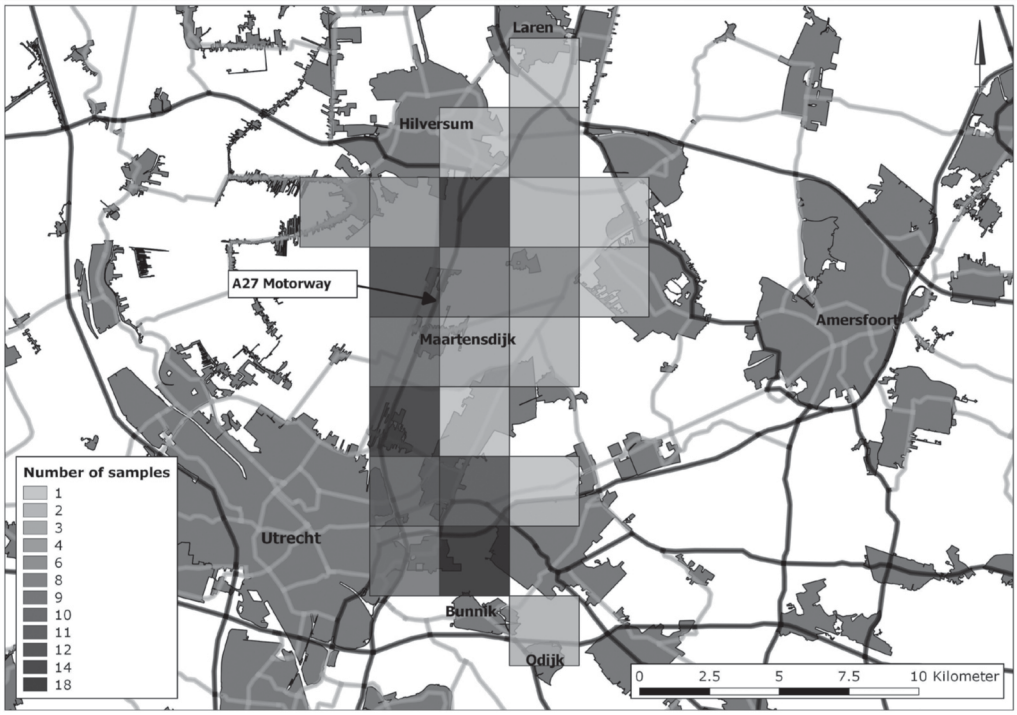

Figure 1. Area where samplesoriginated from illustrated using a grid of 2.5x 2.5 kilometre. Light squaresillustrate areas with few samples, dark squares illustrate areas where most of the samples were collected.

area and surroundings (van Apeldoorn et al. 2006). Badgers are now expanding onto previously thought unsuitable habitat (Zekhuis & Gerrits 2015).As a result of more land becoming inhabited by an increasing badger population, the role of corridors may have shifted from a measure enabling individuals to colonise new territoria, to a measure which connects established territoria among each other.

Measuring genetic differentiation between populations of badgers in the fragmented region of Utrecht was the scope of this study. For this, we used a molecular approach. Tissue from road kills, trapped badgers and faecal samples were used to understand population structure and genetic diversity. Samples were filtered for sample quality and microsatellite analyses of seven different loci were conducted on the selected, good quality samples. Genetic differentiation was tested for populations on either sides of landscape elements that may affect gene flow. Measures in order to reconnect populations, such as corridors, are widely implemented in the study area. The origin of the population and re–connecting measures in place, led to the hypothesis that badger population structure would resemble one connected population.

Methods

Study area

This study was carried out in the Utrechtse Heuvelrug area, Province of Utrecht, the Netherlands. The Utrechtse Heuvelrug is a push moraine formed in the Saale glaciation. The moraine is characterised by sandy soils. These sandy soils change into more clay and peat rich soil as the high land transitions into low land to the east. This transition area of

high sandy soils to low clay rich soils formed the main study area. Tissue samples of badgers were collected between the town of Laren and the town of Odijk, covering ± 20 kilometres. The area between Maartensdijk and Bunnik comprised the study area for collection of faecal samples of badgers in the autumn of 2018 (covering ± 7 kilometres), completely overlapped by the area where tissue samples were collected. Composition of the origin of the samples is illustrated in figure 1, exact locations of road kill and faecal samples are not displayed in order to conceal the exact locations of important badger habitat.

Sampling DNA

Tissue samples were collected from road killed (n=60) and trapped badgers (n=21) between September 2011 and August 2018. Tissue samples consisted of tongue tissue or skin biopsies. Location and date were recorded for all road kills and trapped badgers. Sex and age were recorded for trapped badgers. Faecal samples (n=113) were collected from nine clans between 1 August 2018 and 10 October 2018. During the months of September and October, badgers show an increase in the use of latrines. This increase in activity is more profound in February – March and is related to the mating season (Middleton & Paget 1974, Roper et al. 1986). Collection of faecal samples is therefore performed during these periods as much as possible, as more indiviudals from each clan use latrines more frequently.

Sampling faecal matter is a non-invasive method to collect genetic material from badgers. Faecal samples were collected during the morning. The chance of disturbing badgers in their natural habitat is low during this time of day and human scent has the time to fade before badgers become active again. Faecal matter is also most fresh during this time, as the warm sunlight has not had the time yet to warm up the faeces and speed up the process of breaking down DNA. Badgers place their faeces carefully into small pits, or latrines. Multiple faeces from different individuals could sometimes be found in the same latrine. Tissue samples and faecal samples were collected in tubes containing 96% ethanol. Tissue samples were stored at room temperature, faecal samples were stored at –18°C. A sample of the inner matter of the faeces was collected in order to avoid DNA contamination from other faeces in the same latrine. As territoria boundaries were not exactly known for each clan, latrines were tracked down from the main sett. Latrines close to the main sett were assumed to be primairly used by the corresponding clan. Faecal samples were collected for clans who are connected by natural habitat, connected by corridors or disconnected by roads and towns.

DNA extraction

DNA was extracted using the Qiagen DNaesy Blood & Tissue kit (tissue samples) and Qiagen QIAamp PowerFecal DNA Kit (faeces). Microsatellites were used to study population structure of badgers. Microsatellites are mainly non-coding parts of the genome and are non-susceptible to natural selection, making microsatellites suited for a study of population structure. A total of eight polymorphic badger microsatellite sequences were included in the analysis; Mel102, Mel105, Mel106, Mel109, Mel111, Mel113, Mel117 and Zinc_y_ri (Carpenter et al. 2003, Frantz et al. 2003). Loci were selected for allele sizes (bp) < 250 bp to increase the chance of detection on possible degraded samples. In a first test it appeared that amplification success from faecal samples was lowest for Mel113 locus. Therefore, Mel113 was used to make a first shifting between good and bad quality samples. If Mel113 was not amplified consistently using two PCR replica's, the sample was excluded from further tests. All amplifications were performed using 12,5 µL environmental mastermix 2.0 (life technologies),

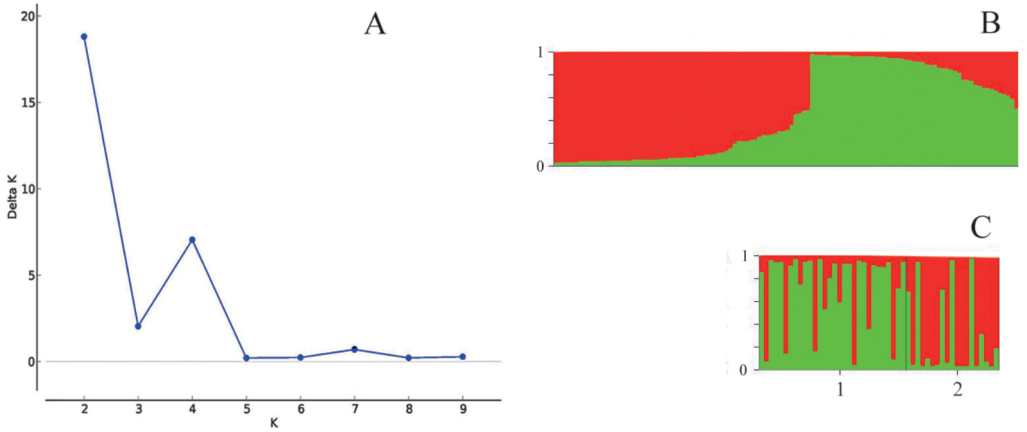

Figure 2. A. To determine population structure, Structure was used to calculate ΔK. Following Evanno et al. (2005) the highest value for ΔK: 2, represented the most accurate estimation for the number of clusters. B. Bars represent population structure for every individual. The red and green colour do not show an abrupt boundary, both populations are therefore not entirely isolated. C. Most distinct difference in population structure was found for both populations on either side of the A27 motoway (1, west of A27 motorway; 2, east of A27 motorway, north of the city of Utrecht).

1 µL of both primers (10 µM), 3 µL template DNA and 7,5 µL DNase free water. Primers were 5'–end labelled with a 6–FAM, Atto550, ATTO532 or ATTO565 dye. PCR was performed for 5 min at 50 °C, 10 min at 95 °C, 55 cycles at 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, followed by a final step at 72 °C for 3 min. PCR was performed in duplo, each marker in a separate reaction. Both PCR reactions of four micro–satellites loci were pooled and length of fragment was evaluated using an ABI 3730DNA Sequencer.

Data analysis

Faecal samples were checked for multiple samples of the same individual per clan, this was later checked among clans. Samples with results for less than four loci were excluded from further analysis. These samples could not accurately be assigned to an individual.

Peaks were called using Geneious 9.1. Geneious enables microsatellite genotyping because it can determine the length of alleles in number of basepairs. Geneious also provides the possibilty to distinguish homozygotes from heterozygotes (Arif et al. 2010). Individual badgers were recognised from multiple samples per clan on the basis of these results. Once the individuals were identified, this same process was repeated among clans. Structure 2.3.4 was used to determine population structure (Pritchard et al. 2000). All individuals from Utrecht were included in this analysis. The total badger population of Utrecht was assumed to be one fully connected population (K=1), a heavily fragmented population (Kmax=9) and all values in between. Length of Burnin Period was set at 5,000, followed by 50,000 replicates. An admixture model with correlated allele frequency among populations was applied for structure analysis. Following Evanno et al. (2005), ΔK was taken as a measure to detect the value of K. Genepop 4.2 was used to calculate population parameters, such as observed–(H_O) and expected heterozygosity (H_E). Weir & Cockerham's (1984) version of F–statistics were used in this study. Here, badgers fallen victim on A27 motorway were excluded as it was unclear which side of the motorway they originated from.

Table 1. Seven loci were included in the genetic analysis. The number of successful analyses (%), total amount of alleles (N), expected heterozygosity (H_E) and observed heterozygosity (H_O) are displayed per locus and per population.

Locus	Total Utrecht population				Utrecht West population				Utrecht East population			
	%	N	H_E	H_O	%	N	H_E	H_O	%	N	H_E	H_O
Mel102	94%	6	0.78	0.67	100%	5	0.68	0.63	91%	6	0.79	0.69
Mel105	97%	6	0.56	0.55	100%	4	0.56	0.58	95%	6	0.56	0.54
Mel106	95%	5	0.52	0.42	100%	3	0.52	0.37	93%	5	0.52	0.45
Mel109	100%	3	0.14	0.14	100%	2	0.07	0.07	99%	3	0.17	0.16
Mel111	90%	5	0.40	0.40	97%	2	0.45	0.55	87%	5	0.37	0.34
Mel113	97%	5	0.56	0.52	97%	3	0.54	0.62	96%	5	0.57	0.48
Mel117	99%	4	0.37	0.38	97%	2	0.24	0.28	99%	4	0.41	0.42
AVG	96%	4.9	0.45	0.42	99%	3.0	0.43	0.44	94%	4.9	0.45	0.41

Results

DNA extration

Extraction of badger DNA, consistent amplification of Mel113, was successful for 98% of the tissue samples (n=81). Analysis was fully successful for 96% of these samples. Tissue samples resulted in 59% males and 41% females. Faecal samples reacted in a different way: 76% of badger faecal samples resulted in the successful extraction of badger DNA. A total of 44 different individuals were successfully identified from the faecal samples, 21 of which were found more than once. Sex ratio was less skewed in faecal samples, 51% males and 49% females.

An extensive search for badger faeces was conducted for five known clans. These territoria are located consecutively, separated by roads. Some of these clans are connected by corridors to neighbouring clans. Between five and thirteen distinct individuals were found for these five known clans. Samples were taken from four other neighbouring clans. Sample size per clan was too small to determine clan size by a mark and recapture analysis (Frantz et al. 2003).

Population structure

Following Evanno et al. (2005) ΔK was taken as a measure to detect the true value of K. The true value of K was found at K=2 (figure 2a). No absolute isolated populations were found, yet clusters of individuals resemble different population structures (figure 2b). The most profound difference in population structure was found between the badger population west of the A27 motorway and east of the A27 motorway, north of Utrecht (figure 2c). A few individuals for both east and west of the A27 motorway match the population structure for the other side of the A27 motorway rather than the side they were found at.

Genetic diversity

The frequency and distribution of alleles and the heterozygosity were used to calculate genetic diversity of the two subpopulations, west of the A27 (hereafter referred to as 'western population') and east of the A27, north of Utrecht (hereafter 'eastern population'). Expected heterozygosity (H_E) and observed heterozygosity (H_O) were calculated for the total population and for each subpopulation (table 1). The eastern badger population

Table 2. F–values for all loci between the two subpopulations, F_{is}: measure of deviation from Hardy–Weinberg within subpopulations, F_{st}: measure of genetic differentiation among subpopulations, F_{it}: measure of deviation from Hardy–Weinberg in total population (also called 'inbreeding coefficient').

Locus	F_{is}	F_{st}	F_{it}
Mel102	0.1145	0.0595	0.1672
Mel105	0.0298	0.0035	0.0332
Mel106	0.1816	–0.0046	0.1778
Mel109	0.0494	0.0103	0.0592
Mel111	–0.0184	0.0361	0.0183
Mel113	0.0791	0.0059	0.0736
Mel117	–0.0402	0.0365	–0.0022
Total	0.0692	0.0219	0.0896

showed a greater diversity in alleles than the western badger population.

All alleles found in the western population were also present in the eastern population. For the total Utrecht badger population H_O was found to be lower than H_E in four of the alleles. In two alleles, H_E and H_O were found to be equal and H_O was found to be higher than H_E. The average heterozygosity for the total Utrecht population was 0.420. This pattern is different between the two subpopulations. The average H_O in the western population (0.437) was found to be slightly higher than the average H_O of the eastern population (0.413), despite a lower number of alleles per loci in the western population (3.0 and 4.9 respectively).

For the total population F values were positive, meaning a deficiency of heterozygotes results in a devation from Hardy–Weinberg equilibrium (Scharloo & van Delden 1987). The measure of deviation from Hardy–Weinberg within subpopulations (F_{is}) overall also shows positive values indicating a deficiency of heterozygotes within the subpopulations. The eastern population showed significant ($P<0.05$) heterozygote deficit for Mel102, Mel105, Mel106 and Mel113. The western population showed significant ($P<0.05$) heterozygote deficit for Mel105 and Mel106. For these alleles more homozygotes were found than expected. More homozygotes for these alleles were also found for the total population (F_{it}). Heterozygote deficits were greater for the total population than within subpopulations.

Following Hartl & Clark (1997) F_{st} values ranging 0–0.05 indicate little differentiation, 0.05–0.15 moderate differentiation, 0.15–0.25 great differentiation and >0.25 very large differentiation. These guidelines were previously also applied in Baird's tapirs (Tapiris bairdii) (Norton & Ashley 2004). The F_{st} value for the badger subpopulations and the total badger population in Utrecht could indicate little differentiation (table 2). The F_{st} value in locus Mel102 indicates moderate genetic differentiation.

Discussion

The badger population in the study area in the Province of Utrecht can be considered as a population consisting of two subpopulations on either side of the A27 motorway. However, neither of the subpopulations were found to be completely isolated populations. The A27 motorway may act as an underlying factor limiting gene flow between two subpopulations. This is in contrast with our initial hypothesis that the population structure of badgers would resemble one connected population.

The Utrecht badger population shows no clear similarity in allele composition to foreign populations such as found in badger populations in England and Switzerland by Frantz et al. (2010b) or Ireland by Guerrero et al. (2018). Average heterozygosity and average number of alleles per locus in the Utrecht population measured over all loci included in this study (0.420; 4.9) appeared to be lower than in the Swiss (0.614; 5.9), English (0.557; 5.4) or Irish study populations (0.461; 6.0). Heterozygosity and allelic diversity are both

indicators for genetic diversity of populations which in turn contributes to fitness and evolutionary potential. High heterozygosity,which is often accompanied by rich allelic diversity, indicates genetically diverse populations with little to no effect of inbreeding on genetic diversity. Low heterozygosity, in fragmented populations, might be caused by genetic drift and limited gene flow. The Utrecht badger population, if compared to study populations in Switzerland, England and Ireland, might therefore have lower fitness and might be more susceptible to loss of genetic diversity as a result of factors such as inbreeding.

Given the limited genetic pool from a small founder population, limited introduction of badgers and supposed little connection between the badger population on the Utrechtse Heuvelrug and Veluwe Nationaal Park, the result of two subpopulations with genetically different structures is consequential. Especially since Van de Zande et al. (2006), who studied badger populations from different regions in the Netherlands and using different loci, found coherence among badger populations. The coherence was in part explained by the relative 'connectiveness' of habitat by corridors. The population west of the A27 motorway contains fewer alleles for every locus in this study and consequently holds a lower gene diversity. The additional alleles in the eastern population might indicate gene flow between the badger population of the Utrechtse Heuvelrugand Veluwe Nationaal Park. However, genetic drift in the western population accompanied with limited gene flow from the eastern population might also be an underlying cause of the difference in alleles between subpopulations. If the latter is part of the driving factor in the genetic differentiation found in this study, then the A27 motorway can be considered a barrier dividing populations, despite efforts to reconnect the fragmented populations.

How corridors mitigate restricted gene flow in the fragmented habitat of badgers appears to be difficult to derive from this study. Factors such as the age of corridors, genetic diversity between generations and the length and complexity of badger dispersal (Roper et al. 2003) for example were not taken into account. Territorial behaviour and disperal behaviour of badgers in relation to corridors might further negatively affect gene flow. Corridors are often completely confined within one territory (Mulder 2016).With dispersing badgers often moving to a neighbouring territory (Roper et al. 2003), gene flow would in most cases only occur via the clan living near a corridor and possibly restraining gene flow.

Several individuals found on one side of the A27 motorway showed a genetic structure resembling that of the individuals on the opposite side of the A27 motorway. Successful colonisations may explain some of these exceptions as one of these concerns a badger who was caught on its sett just southeast of the village of Hollandsche Rading and the A27 motorway. This badger may successfully have migrated to the opposite end of the barrier. Most of the exceptions however (figure 2b) were badgers killed in close proximity to the A27 motorway. It is therefore likely that these badgers crossed the A27 motorway but were killed shortly after. These badgers may therefore unjustly be asigned to a subpopulation.

Collecting faecal samples for genetic population studies on badgers has proven to be a promising method. This method is non-invasive and linking individuals to main setts is found to be possible once accompanied with an elaborate field study for latrines. Faecal samples would be collected from established clans and the link to the area is therefore strong. As most badgers are killed in traffic during periods of high sexual activity, the samples collected from road kills may very well be from dispersing badgers, making it less reliable to link them to a particular area. Badger DNA was successfully extracted out of 76% of the faecal samples. Lammertsma et al. (2006) successfully extracted DNA from the faeces of otter (Lutra lutra) in 18% of the

samples. Wilson et al. (2003) had a 89% success rate in badgers. Contrary to Wilson et al. (2003), not all faecal samples were collected for a consecutive period of time. A higher succes rate is therefore possible once adapting a method wherein latrines are carefully mapped prior to collecting samples and samples are collected when fresh.

As shown by Mulder (2016), roads act as barriers to badgers and consequently, major roads act as territory boundaries. Frantz et al. (2010a) found evidence that a motorway may also restrict badger movement. Corridors therefore do not only provide safe passage, but may also be of importance for communication possibilities between clans in a fragmented habitat. In this study several latrines were often found near corridors. Latrines undisputedly play a major social role in the life of badgers (Roper et al. 1986). They have been described as ways of communication between clans, as ways to attract mates and as ways to defend territories, setts or main foraging grounds. Latrines are assumed to play major roles in establishing territory boundaries. Male badgers were therefore found to show increased use of latrines (Kilshaw et al. 2009). Latrine use in autumn is assumed to be considerably male biased (Roper et al. 1993). The results of this study however show that use of latrines between sexes is nearly equal. This might indicate that latrines play a socially more important role during autumn, when these samples were taken, than previously assumed or that females take as much part in establishing territory boundaries as males. A more comprehensive molecular study, including more seasons, could shed more light on the actual difference in use of latrines between male and female badgers and how this changes throughout the year.

In this study we found two subpopulations on either side of the A27 motorway that differed in population structure and are slightly genetically differentiated, despite efforts to connect badger habitat by several corridors. In order to effectively conserve badger populations, the amount of possibilities for badgers to disperse and therefore opportunities for gene flow to occur may need to increase. Safe passage across barriers in the form of a corridor or 'traffic–calmed areas' (Jaarsma 1997) may be required for every territory alongside significant barriers. Obstacles such as major motorways significantly negatively affect safe passage. The effect of minor roads however should not be underestimated (van Langevelde et al. 2009). Further, we encourage efforts to collect tissue and faecal samples from badgers from different areas and years. Such samples could prove to be crucial to study fluctuations in genetic diversity and thus gene flow between subpopulations, and to be of vital importance to study the mitigating effects of corridors on population structure in the long run.

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Samenvatting

De invloed van barrières op de genetische structuur van dassenpopulaties in een versnipperd landschap

De toename van stedelijk gebied met de daarbij behorendeinfrastructuur zetflora enfauna verder onder druk. Dit geldt ook voor de das (Meles meles). Door fragmentatie van natuurlijk gebied wordt het totale leefgebied verkleind en raken leefgebieden geïsoleerd. De fragmentatie versterkt de effecten van genetische drift. Migratie, en daarmee de uitwisseling van genen (gene flow), wordt bemoeilijkt, waardoor steeds kleiner wordende populaties op zichzelf worden aangewezen. Faunapassages zijn toegepast om geïsoleerde leefgebieden weer te verbinden. Deze faunapassages zijn bewezen, effectieve middelen om verschillende soorten veilig barrières over te laten steken. Het is echter onbekend of faunapassages populaties ook zodanig verbinden dat de uitwisseling van genenweer hersteld wordt. Om de genetische variatie binnen een gefragmenteerde, met faunapassages verbonden, populatie vast te stellen is de genetische structuur van de dassenpopulatie ten noordoosten van de stad Utrecht onderzocht. Aan de hand van genetisch materiaal, afkomstig van dierlijk weefsel en uitwerpselen, is een studie uitgevoerd waarbij microsatellieten zijn gebruikt om individuen te herkennen en genetische variatie te meten. Uit de studie rond de Rijksweg A27 komt naar voren dat de populatiestructuur van dassen aan beide zijden van deze significante barrière opvallend blijkt te verschillen. De populatie ten westen van de A27 wordt ten opzichte van de oostelijke populatie gekenmerkt door enerzijds een vergelijkbare mate van heterozygositeit, maar anderzijds door een lagere diversiteit aan allelen. Ondanks de implementatie van verschillende faunapassages, lijkt de A27 meer dan eerder verondersteld mogelijke migratie te bemoeilijken, en daarmee de uitwisseling van genen, gezien de bestaande verschillen in de populatiestructuur aan weerszijden van de rijksweg.

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