Freie Universität Berlin

Population structure and behaviour of the European hedgehog in an urbanized world

---

Inaugural-Dissertation
to obtain the academic degree
Doctor rerum naturalium (Dr. rer. nat.)

submitted to the Department of Biology, Chemistry and Pharmacy
of Freie Universität Berlin

by

Leon Marian Fynn Barthel
28. March 2019
Berlin

Leibniz-Institut für Zoo- und Wildtierforschung
IM FORSCHUNGSVERBUND BERLIN E.V.

Leibniz-Gemeinschaft

Bridging in Biodiversity Science - BIBS
COLLABORATIVE PROJECT

SPONSORED BY THE

Federal Ministry of Education and Research

Erstgutachter/in:

First reviewer: Dr. Anne Berger

Zweitgutachter/in:

Second reviewer: Prof. Dr. Heribert Hofer

Tag der Diputation 13.09.2019

To my beloved family, who support me and created my childish interest and love of nature.

Dedicated to my father

Bernhard Klein-Barthel *8.3.1954 †12.09.2017

This doctoral thesis was part of the 'Igel in Berlin Project' of the Leibniz Institute for Zoo and Wildlife research within the Bridging in Biodiversity Science (BIBS) Project of The Berlin Brandenburg Institute of Advanced Biodiversity Research (BBIB).

The work was conducted under the supervision of:

Dr. Anne Berger Prof. Dr. Heribert Hofer

Summary

Since humans evolved, they have influenced and changed their environment, which started with hunting pressure and has culminated in climate change. Urbanized areas are arguably the most altered environments on earth, with the process of urbanization transforming natural landscapes in into human-dominated areas. Previous research has found urbanized areas are associated with higher biodiversity, provide refugee habitats for species from various taxa and support high population densities. However, high urbanization can also lead to declining population numbers, local extinction, higher invasion rates of species and potential yet undescribed consequences. Especially the consequences of elevated stressors, which are not exclusive to cities, but are more pronounced than in natural habitats, still need to be elucidated. Therefore model species for the urban living wildlife have to be identified.

The Western European hedgehog (*Erinaceus europaeus*, hereafter hedgehog) could serve as such a model species as it is geographically widespread. These days, hedgehogs are found in higher density close to urbanised areas while its numbers are declining in some areas of its range. Furthermore, hedgehogs might suffer from the densification of urban areas. The construction of infrastructure causes fragmentation and fragmentation can impede the movement of individuals. This fragmentation can influence gene flow between subpopulations when there is no or only restricted access to mates. Hedgehogs, in particular, could be affected by their restricted dispersal and susceptibility to barriers. Thus, the species characteristics and ecology make them an appropriate model species to understand the effect of humans on small ground-dwelling overlooked species in cities. Therefore the genetic diversity and behavioural plasticity of hedgehogs in a suitable study site like Berlin have to be investigated.

In the first genetic study on hedgehogs in a city of this size (~1050 km²), we found, against our expectations, no genetic structure in the population. In a different analysis approach, we could identify ‘family-clan’ structures, which could be an early sign of inbreeding in subpopulations. With our study on the genetic structure of hedgehogs and the identification of new genetic markers, we provide the foundation for future projects to identify the effects of urbanisation on genetic diversity of hedgehogs and evidence-based population management in future. Together with the results of the ecological studies and effects of disturbance and fragmentation on the behaviour of selected hedgehog populations we can draw a better picture of small mammals in cities. For the ecological studies, we developed novel attachment methods in a species-specific manner. We improved methods by considering the welfare of the studied animals, the cost

efficiency and flexibility of the attachment system. This system enabled us to collect spatial and temporal high-resolution data with longer durations than ever before (GPS units). Thus it was possible to follow hedgehogs through an occurring festival in a semi-natural park and in a fragmented zoological garden, identifying population and individual coping strategies, which highlight the behavioural flexibility of hedgehogs.

While we were able to show that research is necessary at both the population level and individual level, without continued monitoring of population genetic and individual behaviours using specialized techniques such as ours, we will never be able to fully understand the greater intricacies of such complex ecosystem as we have in urban centres. We now have the technologies and methods to make evidence-based management decisions and harbour species in cities. It is clear, that cities need to give nature space. As long as flora, fauna and funga have enough connections between fragmented "green" areas it might help to sustain healthy populations and could even provide source populations in the process of rural restoration. However, it is also clear that cities are not enough to harbour all wildlife alone in the long-term, especially if the remaining green spaces vanish and cities continue to condense.

Zusammenfassung

Seit Beginn der Menschheit nimmt der Mensch Einfluss auf seine Umwelt, was mit dem Jagddruck anfing und sich heute durch sekundäre Einflüsse wie Klimawandel auswirkt. Urbanisierte Gebiete sind wohl die am stärksten veränderten Umgebungen der Erde. Der Prozess der Urbanisierung verwandelt Naturlandschaften auf extreme Weise in vom Menschen dominierte Gebiete. In früheren Studien wurde festgestellt, dass urbanisierte Gebiete mit höherer Biodiversität verbunden sind, Rückzugsräume für Arten aus verschiedenen Taxa bieten und hohe Populationsdichten unterstützen können. Eine starke Verstädterung kann jedoch in anderen Fällen auch zu sinkenden Populationsdichten, zu lokalem Aussterben, höheren Invasionsraten und noch nicht bekannten Folgen führen. Insbesondere die Folgen erhöhter Stressoren, wie zum Beispiel Verschmutzung, Lärm und höheren Temperaturen im Vergleich zu ländlichen Lebensräumen müssen noch geklärt werden. Daher müssen Modellarten für die städtische lebende Tierwelt identifiziert werden, die nicht nur verschiedene Taxa sondern auch verschiedene Charakteristika mit sich bringen.

Der Braunbrustigel (*Erinaceus europaeus*, hiernach auch Igel) mit seiner weiten geographischen Verbreitung könnte eine solche Modellart sein. Igel werden heutzutage in höheren Dichten in der Nähe von urbanisierten Gebieten gefunden und nehmen in Teilen ihres Verbreitungsgebietes ab. Es könnte sein das der Igel unter der voranschreitenden Verdichtung der urbanen räume leidet, denn durch seine charakteristische Morphologie ist er durch Barrieren, die in Städten auftreten, unmittelbar betroffen. Diese Fragmentierung behindert die Bewegung von Individuen. Zusammen mit der Verhaltensbiologie der Igel könnte so die genetische Struktur der Igelpopulation in Städten beeinflusst werden. Mit seiner eingeschränkten Ausbreitung und Anfälligkeit für Barrieren könnte der Igel als Modellart für andere bodenlebende Säugetierarten sein, die sonst übersehen werden.

In der ersten genetischen Studie an Igeln in einer Stadt dieser Größe (~1050 km²), fanden wir - entgegen unseren Erwartungen - keine genetische Struktur in der Igel Population. In einem anderen Analyseansatz konnten wir „Familien-Clan' -Strukturen identifizieren, die ein frühes Zeichen für Inzucht in Subpopulationen sein könnten. Mit unseren Studien zur genetischen Struktur von Igeln und zur Identifizierung neuer genetischer Marker legen wir den Grundstein für zukünftige Projekte. Dadurch können die Auswirkungen der Verstädterung auf die genetische Vielfalt von Igeln besser verstanden werden und, wenn nötig, kann evidenzbasiertes Populationsmanagement initiiert werden. Diese Informationen und die Ergebnisse der

ökologischen Studien, in welchen wir den Einfluss eines Musikfestivals und der Fragmentierung der Stadtstruktur auf das Verhalten von ausgewählten Igelpopulationen untersuchten, können dabei helfen, ein vollständigeres Bild von Igeln in Städten zu liefern. Für die ökologischen Studien haben wir eine neuartige Anbringungsmethode artspezifisch entwickelt. Dabei verbesserten wir das Wohlbefinden der untersuchten Tiere, die Kosteneffizienz und die allgemeine Flexibilität bei Studien im Freiland. Dieses System ermöglichte es uns räumlich und zeitlich hochauflösende Daten zu sammeln und dabei den Tieren länger als zuvor mit GPS Geräten zu folgen. So war es möglich, Igel durch ein stattfindendes Festival und in einem fragmentierten zoologischen Garten zu verfolgen, wobei die Reaktion der Population im Allgemeinen und die individuellen Strategien identifiziert werden konnten, die die Verhaltensplastizität von Igeln unterstreichen.

Während wir zeigen konnten, dass Studien sowohl auf Population als auch auf individueller Ebene notwendig sind, ist es ohne durchgehendes Monitoring der genetischen Diversität und des individuellen Verhaltens der Population nicht möglich die Feinheiten eines solch komplexen Ökosystems wirklich verstehen zu können. Wir leben in einem Zeitalter, in welchem wir über Technologien und Methoden verfügen, um Wissen zu sammeln und daraus evidenzbasierte Managemententscheidungen zu treffen. Dadurch wird es uns möglich sein, zu naturfreundlichen Städten beizutragen. Es ist klar, dass Städte der Natur Raum geben müssen. Solange Flora, Fauna und Funga über genügend „grüne“ Verbindungen verfügen, können Städte möglicherweise gesunde Populationen aufrechterhalten und sogar Quellbestände für die Wiederansiedlung wiederhergestellter ländlicher Gebiete liefern. Denn auch dies ist klar: auf lange Sicht reichen Städte nicht als Lebensraum für die Arten aus, vor allem wenn noch existierende Grünflächen verschwinden und sich die Städte weiter verdichten.

.

Content

Summary i

Zusammenfassung iii

List of figures ix

List of tables xiv

Introduction: A changing and urbanizing world 1

The Western European hedgehog (*Erinaceus europaeus*) is an elusive species with potential as a model species 5

Objectives & purpose of study 6

Structure of this thesis 7

Manuscripts and Author contribution 8

1 Unexpected gene-flow in urban environments: the example of the European hedgehog 10

1.1 Abstract 10

1.2 Introduction 11

1.3 Materials & Methods 12

1.4 Results 15

1.5 Discussion 20

1.6 Conclusions 22

1.7 Acknowledgements 28

1.8 References 28

2 Application of an SSR-GBS marker system on an investigation of European Hedgehog species and their hybrid zone dynamics 33

2.1 Abstract 33

2.2 Introduction 34

2.3 Material and methods 37

2.3.1 Sampling and DNA isolation 37

2.3.2 Marker development 38

2.3.3 SSR-GBS amplicon library preparation 39

2.3.4 Sequence data extraction 41

2.3.5 Allele definition 43

2.3.6 Population genetics analysis 45

2.4 Results 46

2.4.1 Maker development 46

2.4.2 Sequence analysis and genotyping 46
2.4.3 Marker variability 47
2.4.4 Comparison between genotyping approaches and species-specific primers 50
2.4.5 Genetic structure 52
2.5 Discussion 55
2.5.1 Marker specificity 56
2.5.2 Better resolution 57
2.5.3 Simplification of the procedure 58
2.5.4 Better reproducibility and easier analysis 58
2.5.5 Phylogeographic implications 61
2.5.6 Importance of the museum collections 62
2.6 Acknowledgements 62
2.7 References 62
3 An easy, flexible solution to attach devices to hedgehogs (*Erinaceus europaeus*) enables long-term high resolution studies 70
3.1 Abstract 70
3.2 Introduction 71
3.3 Methods 73
3.3.1 Study area & animals 73
3.3.2 Backpack attachment 73
3.3.3 Fitting and removing of the backpack 75
3.3.4 Statistical analyses 76
3.4 Results 76
3.5 Discussion 78
3.6 Acknowledgements 82
3.7 References 82
4 Distinguishing spatial from temporal effects in disturbance biology: Hedgehogs in the urban matrix of habitat fragmentation and noise pollution 87
4.1 Abstract 87
4.2 Introduction 88
4.2.1 Predictions 90
4.3 Methods 92
4.3.1 Fieldwork 92
4.3.2 Analyses 94

4.4 Results ... 98
4.4.1 Movement data ... 98
Behaviour ... 100
Nesting Behaviour ... 101
4.5 Discussion ... 102
4.5.1 Movement Data ... 103
4.5.2 Behaviour ... 104
4.5.3 Nesting ... 106
4.5.4 Conclusion and outlook ... 106
4.6 Acknowledgements ... 107
Founding ... 107
4.7 References ... 108
5 Music festival makes hedgehogs move: How individuals cope behaviourally in response to human-induced stressors ... 114
5.1 Introduction ... 115
5.2 Materials and Methods ... 116
5.2.1 Study Area ... 116
5.2.2 Semi-experimental design ... 117
5.2.4 Study animals and logger attachment ... 117
5.2.5 Logger setup ... 118
5.2.6 Data Analysis ... 118
5.3 Results ... 122
5.3.1 Spatial Results ... 122
5.3.2 Model evaluation for behaviour prediction ... 123
5.3.3 Behaviour prediction ... 123
5.3.4 Behaviour budget ... 124
5.3.5 Daily activity pattern ... 124
5.3.6 ODBA analysis ... 126
5.3.7 Stress detection ... 127
5.4 Discussion ... 128
5.4.1 Spatial behaviour ... 128
5.4.2 Behaviour analysis ... 129
5.4.3 Daily activity pattern ... 130
5.4.4 ODBA analysis ... 131

5.4.5 Stress detection ... 131

5.5 Conclusions ... 132

5.6 Acknowledgements ... 133

Funding 133

5.7 References ... 134

General discussion ... 137

Genetic of hedgehogs in urban systems ... 137

Movement and behaviour ... 139

Past, present and future ... 141

Acknowledgments ... 143

References from introduction and general discussion ... 144

1 Supplementary information Chapter 1: Unexpected gene-flow in urban environments: the example of the European hedgehog ... 153

2 Supplementary information Chapter 2: Application of an SSR-GBS marker system on an investigation of European Hedgehog species and their hybrid zone dynamics ... 165

4 Supplementary information Chapter 4: Distinguishing spatial from temporal effects in disturbance biology: Hedgehogs in the urban matrix of habitat fragmentation and noise pollution182

5 Supplementary information Chapter 5: Music festival makes hedgehogs move: How individuals cope behaviourally in response to human-induced stressors ... 202

Affirmation for the Doctoral Thesis/ Selbstständigkeitserklärung ... 204

List of figures

Figure 0.1: 'Urban environmental conditions viewed in the context of spatial scales and timescales. The location of the text indicates the most prevalent scale of impact. The horizontal arrows indicate the range of temporal scales and the vertical arrows indicate the range of spatial scales that may be impacted. Biotic interactions include competition, predation, parasitism, mutualisms, facilitation, etc. Physical disturbances have been divided into two broad categories (asterisks): Acute disturbances are generally individual events located within relatively discrete places in time and space (e.g., vandalism, storms) and chronic disturbances are generally more repetitive events that occur over longer timescales and possibly broader spatial scales (e.g., trampling).' From McDonnell and Hahs 2015 2

Figure 1.1: Map of Berlin and its surroundings and showing the locations from 139 out of 143 samples (four samples not shown because outside of the map) 13

Figure 1.2: Map of Berlin and its surroundings and showing the locations from 64 out of 65 unrelated samples (one sample not shown because it was located 70 km north of Berlin), later by Structure identified as one cluster 19

Figure 1.3: Taking a saliva sample from a hedgehog in Treptower Park. Picture by Rohit Chakravarty 32

Figure 2.1: Summary of sequence analysis and genotyping approach. The top left panel shows an overview of the method. The right and bottom panels show decision trees concerning: allele call based on length (1), stutter control step (2), detection of SNP genotypes (3). L1 and L2 correspond to the two most frequent lengths found per sample and marker, while f(L1) and (L2) to their frequency. f(b1), f(b2), and f(b3) correspond to the frequencies of the most, second most and third most frequent nucleotides per position, respectively. f(bcomb1), f(bcomb2), and f(bcomb3) correspond, respectively, to the frequencies of the most, second most and third most frequent nucleotides combinations of two or more potential SNPs.......... 42

Figure 2.2: Number of reads per amplicon length. The left panel shows unambiguous heterozygote genotypes for tri-, tetra-, and pentanucleotide motifs. The right panel shows examples matching the three cases of the automatic stutter control: Case I, two alleles with a length difference above the repetition motif length; case II, two alleles with length difference equal to the motif length, whose the frequency of the shorter is higher than the longer one; case III, two alleles with length difference equal to the motif length, whose the frequency of the shortest allele is more than 75% of the longer one. Green bars correspond to amplicon lengths chosen as alleles by the genotyping method. Numbers above each bar indicate the allele length.

The line above each graph indicates the chosen genotype and the corresponding number of reads supporting it 44
Figure 2.3: Number of alleles shared among individuals shown as the number of alleles (*y*-axis) in dependence to the number individuals that share one allele (*x*-axis). White and grey bars represent alleles called using sequence length information, respectively. The comparison includes the final 41 markers for all 82 individuals 50
Figure 2.4: Boxplots describing variability and genetic diversity measurements per marker. Left panel using different allele calling approaches: sequence length (L) and sequence information (S). Right panel using different markers sets: *E. europaeus*-specific primers (E) and *E. roumanicus* species primers (R). *p*-Values correspond to *t*-tests comparing differences in averages between genotyping methods and markers sets 51
Figure 2.5: Boxplots describing pairwise distance between samples. Left panel using different allele calling approaches: sequence length (L) and sequence information (S). Right panel using different markers sets: *E. europaeus*-specific primers (E) and *E. roumanicus* 52
Figure 2.6: Principal coordinates analyses from matrix with genotypes called based on sequence information. Top: PCoA with complete dataset. Middle: PCoA with only *E. europaeus* samples. Bottom: PCoA with *E. roumanicus*. In the analysis for all samples, the samples; errata Error: *E. europeaus* Correction: *E. europaeus*.. 53
Figure 2.7: Structure analysis for all three datasets (All samples, only *E. europaeus*, only *E. roumanicus*) considering all markers and alleles called based on sequence information. Only results from K = 2 until the optimum are shown 55
Figure 3.1: (A) A complete backpack glued to the hedgehog's spines including the back plate consisting of firmly glued fabric (blue) and loop strap (black below the blue fabric), the wires (green), the GPS device (transparent shrink tube) and the VHF transmitter (black). (B) The back plate system from above; scale numbers indicate cm. Photograph: Leon M.F. Barthel 75
Figure 3.2: Boxplots of body mass of hedgehogs on the first day of deployment (Start) and on the last day of deployment (End). Central line: median, x: location of mean, whiskers: 1.5 times the interquartile range, circle: values more extreme than 1.5 time's interquartile range around the median. 78
Figure 3.3: Hedgehog with the back plate system 86
Figure 4.1: statistical study design, in green the control from the pre-festival period in the Treptower Park is compared to the festival dataset (reddish) and the fragmented dataset of the Tierpark (blue), arrows indicate statistical comparisons... 95

Figure 4.2: Results of the GPS data A: MCP 95 indicating Measurements for used area dots representing females, triangles males; B: mean of speed [m/s] over both sexes against treatment C: mean of search intensity [m/(m²*d)] over both sexes against treatment; dots/triangles indicate mean values; whiskers are confidence interval, D: Distribution of absolute turning angles showing one of the subsamples that were tested 99
Figure 4.3: Centroid distribution circle/reddish = pre.-festival, triangle/bluish = festival, density shows kernel density estimate, all y-axes in the density plots show the same range. 100
Figure 4.4: Behaviour and circadian rhythmic of hedgehogs in the context of acute/ transient and permanent habitat changes. A; odd value of balled up behaviour B; relative part of walking behaviour C; resting behaviour D; Degree of functional coupling; whiskers = 95% family-wise conf.int. 101
Figure 4.5: Nest utilization period probability of both datasets dataset A dataset B separately for sex. Dataset A: pre/after-festival and festival phase of 17 hedgehogs (nine males, eight females) p values coming from Log-rank test females: Log-Rank, N=88, Mantel, p-value=0.83; males: Log-Rank, N=156, Mantel, p-Value=0.02. Dataset B: fragmentation x-axis shorten for comparison. 102
Figure 4.6: Hedgehog squeezing under the VIP Container of the Lollapalooza festival 113
Figure 5.1: mean values with a confidence interval for nightly Kernel density estimation 50 % (50KDE) areas of eight hedgehogs in the pre-festival and festival phase (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour. 122
Figure 5.2: Nest utilization period probability of females and males during pre/after-festival and festival phase of 17 hedgehogs (nine males, eight females) p values coming from Log-rank test females: Log-Rank, N = 88, Mantel, p-value = 0.83; males: Log-Rank, N = 156, Mantel, p-Value = 0.02. 123
Figure 5.3: Mean values with confidence interval for the diurnality index DI of eight hedgehogs in the pre-festival and festival timeframe (significant differences are marked by *, Wilcoxon test cf. Supplementary Table 5.1). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour. 125
Figure 5.4: Mean values with confidence interval for the time span between activity onset and civil dusk (TSdusk) of eight hedgehogs in the pre-festival and festival timeframe (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour. 126

Figure 5.5: Mean values with confidence interval for the Overall Dynamic Body Acceleration (ODBA) of seven hedgehogs in the pre-festival and festival timeframe (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour. 127
Figure 5.6: Mean values with confidence interval for the Degrees of Functional Coupling (DFC) of eight hedgehogs in the undisturbed and disturbed timeframe (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour. 128
Figure 0.2: The feedback system between environment, behaviour and biodiversity. Changes in environmental conditions induce behavioural responses according to the reaction norm of the individual, which in turn affects species interactions, population dynamics, evolutionary processes and, ultimately, biodiversity. Changes in population dynamics, evolutionary processes and species interactions, in turn, affect behaviour, resulting in a complex network of feedback loops. From (Tuomainen and Candolin, 2011) 140
Supplementary Figure 1.1: Map of all sampling locations (N= 65), displaying the distribution of individual genotype Q-values (STRUCTURE analysis results), values in-between are interpolated. Left: data for cluster 1, center: data for cluster 2, right: data for cluster 3. 153
Supplementary Figure 1.2: Pairwise relatedness after Queller and Goodman (QGEst) of sampled genotypes (Ind1 and Ind2) before (left) and after (right) removing related genotypes r > 158
Supplementary Figure 2.1: Correlation between geographical and genetic distance among individuals. Distances was calculated among individuals because of lack of population sampling. Upper graph includes individuals from *E. europaeus* only while the bottom one contains individuals from *E roumanicus*. errata Error: *E. europeaus* Correction: *E. europaeus* 179
Supplementary Figure 2.2: Structure analyses for all three datasets (all samples, only *E. europaeus,* only *E. roumanicus*) considering all markers and alleles called based on both sequence and length information. Only the results for the optimum K values are shown: K=2 for all samples; K=3 for *E. europaeus*; and K=5 for *E. roumanicus*. 180
Supplementary Figure 2.3: Structure analyses for all three datasets (all samples, only E. europaeus, only E. roumanicus) considering markers developed specifically for *E. roumanicus* (R) and *E. europaeus* (E) and alleles called based on sequence information. Only the results for the optimum K values are shown: K=4 for E. europaeus; K=3 for E. europaeus 181
Supplementary Figure 4.1: Multiple Comparisons of Means for the MCP95 183
Supplementary Figure 4.3: Results of the KDE50.. 183
Supplementary Figure 4.2: speed pairwise comparison of treatments 184

Supplementary Figure 4.3: all turning Angels of all hedghogs 185
Supplementary Figure 4.4: Raw data latitude of the centroid values per day over time construction work started at the 28th of August and ended on the 16th September...................... 186
Supplementary Figure 4.5: Rawdata longitude of the centroid values per day over time construction work started at the 28th of August and ended on the 16th September...................... 186
Supplementary Figure 4.6: left longitudinal data, right: latidudinal data.. 187
Supplementary Figure 4.7: mean proportion of balling up behaviour against treatments, whiskers indicate confidence interval.. 188
Supplementary Figure 4.8: mean proportion of walking behaviour against treatments, whiskers indicate confidence interval.. 189
Supplementary Figure 4.9: mean proportion of behaviour identified as resting against treatment 190
Supplementary Figure 4.10: left: estimates in pairwise comparisons; whiskers indicate 95% confidence interval right mean proportion of behaviour not classified other, whiskers indicate confidence interval.. 191
Supplementary Figure 4.11: Estimates of Degree of Functional Coupling pairwise comparison 192
Supplementary Figure 4.12: Empirical Cumulative Distribution Function of Distance to the next path, indicating the fragmentation of the habitat.. 194

List of tables

Table 1.1: Indices of ten microsatellite loci across the 143 unique genotypes................................ 17
Table 1.2: Results of both clustering algorithms (Structure, Baps) and their assignment for each sample... 23
Table 2.1: Average, across used loci, of amplification success shown as percentage of missing data and average variability measures: N_a—number of alleles, H_O—observed heterozygosity, H_E—expected heterozygosity, and PIC—polymorphism information content. Values in brackets correspond to minimum and maximum values. Values calculated based on sequence information are represented by the superscript 'S' while the ones based on length information by 'L'. Statistics were calculated based on different markers and samples sets... 49
Table 3.1: Characteristics of all study animals: ID, sex, body mass at start and end of the experiment, body mass change number of deployments, duration of complete deployment, and, for illustrative purposes, the relative mass for a 30 g back plate device combination................... 77
Table 5.1: Animal identification number (ID), sex and body mass (at the date of logger attachment) of the studied hedgehogs.. 117
Table 5.2: Recall and precision [31] of the hedgehog model.. 123
Table 5.3: Changes in the behaviour during the festival phase in comparison to the pre-festival phase. Values represent the odds ratio for every behaviour separately with the corresponding p-value calculated with the Fisher's Exact Test. Odds ratios smaller than 1 indicate a decrease in the behaviour in the festival phase while an odds ratio greater than 1 indicate an increase........ 124
Supplementary Table 1.1: Origin of samples (N = 143)... 153
Supplementary Table 1.2: Unique genotypes (N = 143) of hedgehogs in Berlin across 10 loci. 159
Missing values are indicated by '0'.Supplementary Table 1.3: Unique unrelated genotypes (N = 65, r < 0.5) of hedgehogs in Berlin across 10 loci.. 162
Supplementary Table 2.1: Samples used with information of location of origin, species identification, starting material for DNA isolation, institution providing the sample, and coordinates. For some samples coordinates were not available (NA). 165
Supplementary Table 2.2: Complete list of all primers designed with the following information: from which species they were designed (Species), in which primer mix they were included in the multiplex PCR (Mix), repetition motif (Motif), number of times it was repeated in the original sequence (Nr. Repeats), primer sequence (Forward and Reverse) and allele length variation (Amplicon length variation). 168

Supplementary Table 2.3: Descriptions and procedures used in the in-house-scripts. We used the scripts one after the other and information concerning how this was done can be found in the material and methods. The scripts 1 to 2 can be run sequentially using the wrapper *microsatPip*. 171

Supplementary Table 2.4: Amplification success shown as percentage of missing data and variability measures: Na – number of alleles, HO – observed heterozygosity, HE – expected heterozygosity and PIC – polymorphism information content. Values calculated based on sequence information are represented by the superscript S while the ones based on length information by L. The last seven markers separated from the remaining ones correspond to the markers excluded for the complete analyses either by excess of missing data or lack of variation. 177

Supplementary Table 4.1: Linear model, outcome of ANOVA and results of the multiple comparisons of MCP95 182

Supplementary Table 4.2: Linear model, outcome of ANOVA and results of the multiple comparisons of KDE50 183

Supplementary Table 4.3: Linear model, outcome of ANOVA and multiple comparisons for speed 184

Supplementary Table 4.4: Loop for permutation: example for the comparison of the pre-festival and festival data using the ks.test 185

Supplementary Table 4.5: Linear model and outcome of ANOVA of longitudinal data and latitudinal 187

Supplementary Table 4.6: Linear model, outcome of ANOVA and results of the multiple comparisons for balled up behaviour 187

Supplementary Table 4.7: Linear model, outcome of ANOVA and results of the multiple comparisons of walking behaviour 188

Supplementary Table 4.8: Linear model, outcome of ANOVA and results of the multiple comparisons of resting behaviour 190

Supplementary Table 4.9: Linear model, outcome of ANOVA and results of the multiple comparisons of other (not identified) behaviour 191

Supplementary Table 4.10: Nesting details 193

Supplementary Table 4.11: Nest protocol 195

Supplementary Table 4.12: Nest survival raw data Treptower Park 2016 196

Supplementary Table 4.13: Table Nesting Data from Tierpark 2017 200

Supplementary Table 5.1: Overview over Wilcoxon test results for the different parameters to compare daily values of pre-festival period with them of festival period. Significant differences are marked by bold font. KDE50 = 50% Kernel Density Estimation , DI = Diurnality Index, TSdusk = Time Span between activity onset and civil dusk, ODBA = Overall Dynamic Body Acceleration, DFC = Degree of Functional Coupling 202
Supplementary Table 5.2: Results for the kde 50 for all individuals. Undisturbed corresponds to the pre-festival phase, disturbed corresponds to the festival phase 203

Introduction: A changing and urbanizing world

We are living in a changing world. Since humans evolved they have changed their environment, which started with hunting pressure and has culminated in climate change. During our evolution, humans have congregated in settlements, from villages associated with increasing agriculture and trade, to rapidly expanding megacities. Urban areas are growing worldwide (Grimm *et al.*, 2008) and we have already passed the point where more than 50 % of humankind live in urban areas (UN (United Nations), 2018). Urbanized areas are arguably the most altered environments on earth, with the process of urbanization transforming natural landscapes in extreme ways into human-dominated areas (Seto, Güneralp and Hutyra, 2012).

Urban ecology is a relatively recent, but burgeoning field of ecology (Magle *et al.*, 2012; McDonald, 2016). As urban centres provide a new and altered environment for wildlife and plants, it has been necessary to ask new questions and understand the ecology of species in a new way (Sutherland *et al.*, 2013; McDonald *et al.*, 2018). These environmental changes are acting and interacting which each other along a range of spatial and temporal scales, which can affect wildlife and may force adjustments and adaptation (Figure 0.1, McDonnell and Hahs 2015; Grimm et al. 2008). Trying to grasp the various functional and ecological differences to the 'natural' non-urbanized world has led to new concepts and has resulted in the creation of terms like 'urban avoiders', 'urban adaptors' and 'urban exploiters' (Blair, 1996; McKinney, 2002; Fischer *et al.*, 2015). The use of these novel terms has been debated, therefore the employment of long-established, widely applicable terms like 'long-', 'short-distant migrants' and 'residents', is preferable (McDonnell and Hahs, 2015). Urban ecology is not solely the study of wildlife in urbanized areas alone, but also includes essential factors such as the anthropogenic, social and human health aspects of living in an urbanized environment (Seto, Parnell and Elmqvist, 2013). Previous research has found urbanized areas are associated with higher biodiversity compared to rural areas and provide refugee habitats for species from various taxa and support high population densities (Ives *et al.*, 2016; Threlfall *et al.*, 2017; Kowarik and von der Lippe, 2018). However, high urbanization can also lead to declining population numbers, local extinction, higher invasion rates of species and potential yet undescribed consequences (von der Lippe and Kowarik, 2006; Duncan *et al.*, 2011; Beninde, Veith and Hochkirch, 2015; Gaertner *et al.*, 2017). Especially the consequences of elevated stressors, which are not exclusive to cities, that may be more pronounced than in natural habitats, still need to be elucidated (Beninde, Veith and Hochkirch, 2015; McDonnell and Hahs, 2015; Threlfall *et al.*, 2016; Johnson and Munshi-South, 2017; Kowarik and von der Lippe, 2018).

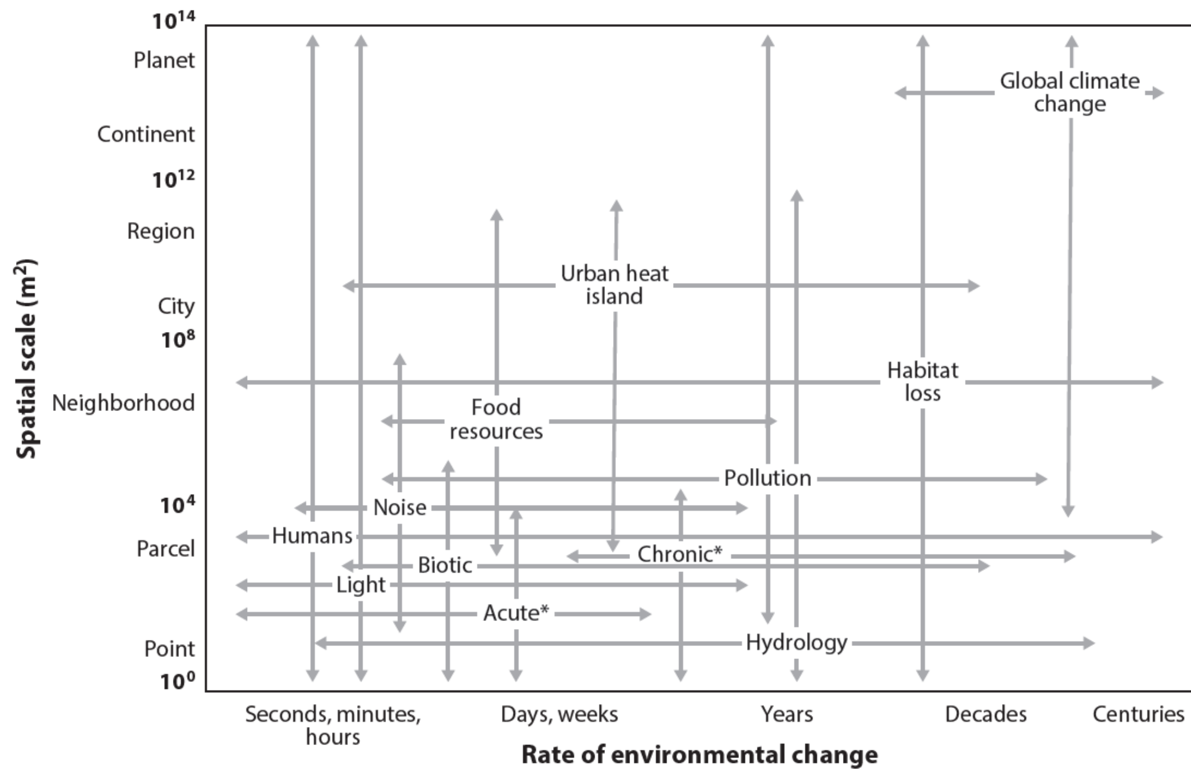


Figure 0.1: 'Urban environmental conditions viewed in the context of spatial scales and timescales. The location of the text indicates the most prevalent scale of impact. The horizontal arrows indicate the range of temporal scales and the vertical arrows indicate the range of spatial scales that may be impacted. Biotic interactions include competition, predation, parasitism, mutualisms, facilitation, etc. Physical disturbances have been divided into two broad categories (asterisks): Acute disturbances are generally individual events located within relatively discrete places in time and space (e.g., vandalism, storms) and chronic disturbances are generally more repetitive events that occur over longer timescales and possibly broader spatial scales (e.g., trampling).' From McDonnell and Hahs 2015

These days, several projects have shown that some animals (e.g. foxes, wild boars, squirrels, flying foxes, racoons and house mice) are able to cope and coexist with humans in cities (McKinney, 2002; Santini *et al.*, 2018). Theories like the refugee species concept, which posits that species live in suboptimal habitats because suitable ones are not available (Kerley, Kowalczyk and Cromsigt, 2012; Kuemmerle *et al.*, 2012; Stirnemann *et al.*, 2017), suggesting that the wild species in cities could struggle with a compromise solution. Therefore, we need to understand if these species sustain stable populations. More specifically, we have to identify the evolutionary processes driving the ability for animals to survive or thrive in urban habitats and remove or mitigate possible threats. It is essential to investigate this for a large number of species with various behavioural, reproductive, morphological and ecological characteristics. Only after obtaining an understanding of the challenges of these species living in urban environments, will we be able to protect the needed resources and sustain or create optimal habitats for the flora and fauna in our cities (Magle *et al.*, 2012).

Urbanization and urban expansion lead to increased infrastructure, which divides formerly connected landscapes into separate patches (Doncaster and Dickman, 1987; Braaker, Moretti, *et*

al., 2014). This habitat fragmentation effects animal movement, behaviour and the ability to retain requirements for successful reproduction and ultimately secure a sufficient gene flow (Magle *et al.*, 2012; Guneralp and Seto, 2013). Animal movement is affected by barriers, unsuitable habitats and landscapes of fear, all of which impedes movement and dispersal (Wauters *et al.*, 1994; Bleicher, 2017; Soanes *et al.*, 2018; Tucker *et al.*, 2018). If species are able to explore new habitats or patches the limited influx of animals or lack of mates can create bottlenecks, inbreeding and thus lower, for example, the diversity of major histocompatibility complex (MHC) genes and general variation of the population (Ellstrand and Elam, 1993; Noël *et al.*, 2007; Belasen *et al.*, 2019). In the new field of 'urban conservation genetics' the reports for various taxa are between stable and reduced genetic variability which could influence the resistance and resilience of species (Noël and Lapointe, 2010; Munshi-South and Nagy, 2014). Moreover, in urbanized areas the increasing density and height of buildings decrease the permeability for urban wildlife even further. With buildings and roads comes sealed surfaces and changed airflow, which can lead to changes in microclimate and eventually generate the so-called urban heat island effect. The local warmer temperatures in cities, especially in summer and winter, may create a range of stressors, causing positive or negative impacts on ourselves as humans and animals living in cities (Mills, 2008; Seto, Parnell and Elmqvist, 2013; Li, 2018).

In this highly altered environments, urban green spaces can create refuge habitats for a range of species (Ansell, Baker and Harris, 2001; Bateman and Fleming, 2012; Haigh *et al.*, 2017). For some species, public parks, cemeteries and abandoned lands are part of their foraging habitats or stepping-stones connecting suitable habitats in their home ranges, whereas others completely rely on these urban green spaces throughout their whole life cycle (Braaker, 2012; LaPoint *et al.*, 2015).

Finally, the presence of humans and their associated factors like traffic, light, noise, pollution and management of the remaining green patches is a constantly influence the wildlife species and ultimately result in the creation of a novel complex ecosystem (Grimm *et al.*, 2008; Kowarik, 2011; Seto, Parnell and Elmqvist, 2013; McDonnell and Hahs, 2015). These potential stressors can not only have lasting effects on humans (Burton, 1990; Díaz *et al.*, 2006), but also on animals living in urban environments (Ditchkoff, Saalfeld and Gibson, 2006; Lowry, Lill and Wong, 2013; Wong and Candolin, 2015; Johnson and Munshi-South, 2017). The important question is whether animals are able to keep up with the rapid changes their habitats undergo, in order to conserve the sustainability and integrity of the population. While the swift extinction of species in this system can be observed quite easily, it is hard to identify wherever animals are constantly

stressed and disturbed and thus declining in numbers (Diamond, 1988; Duncan *et al.*, 2011). However, it is known that stress leads to reduced reproductive success of individuals, thus could play a role in urban environments, while we could assume animals are able to habituate to environments the constantly changing environments of urban habitats or the remarkable difference to natural habitats could lead to chronic stress (Hofer and East, 1998). Chronic stress may influence metabolism and energetics of individuals, usually measured using heart rate or hormonal responses such as raised concentrations of epinephrine or glucocorticoids (Dhabhar *et al.*, 1996; Wingfield, Jacobs and Hillgarth, 1997; Sapolsky, Romero and Munck, 2000; Wikelski and Cooke, 2006). Chronobiological studies are also able to detect disturbance (Scheibe and Gromann, 2006; Berger, 2011). Perturbations in biological rhythms are detectable. Sudden differences in the environment destroy the inner rhythm and are detectable if a baseline behaviour and activity rhythm of the individual is available (Berger, 2011). This is important because it seems that human modifications alter animal behaviour more than natural causes (Wong and Candolin, 2015). Therefore in urbanized areas, more exploratory phenotypes seem to be present; where animals change their activity patterns or adjust their movement and sociality (Bateman and Fleming, 2012; Miranda *et al.*, 2013; Sol, Lapiedra and González-Lagos, 2013; Breck *et al.*, 2019). All these influences ultimately lead to selection pressure on Darwinian fitness factors including reproduction, diversity and survival (Dickman and Doncaster, 1988; Hofer and East, 1998).

In small and ground-dwelling mammals in urban habitats, the effect of habitat fragmentation and the resultant reduction in dispersal and access to mates has an important effect on genetic diversity (Doncaster and Dickman, 1987). With commonly shorter generation times and small dispersal capacity or range size, these animals are likely to be more affected, than animals that can move faster, fly or otherwise overcome physical barriers present in urban habitats (Dickman and Doncaster, 1988). It has already been shown that some species are capable of adapting to the urban environment (Johnson and Munshi-South, 2017) but we need a broader range of model species to understand the complete impact of urbanization on biodiversity and to detect the general pattern. More specifically, there has been an overall lack of investigation on small cryptic species as their populations at first glance often appear to be thriving in cities. Yet the impacts of urbanization on these populations remain understudied or entirely overlooked.

The Western European hedgehog (*Erinaceus europaeus*) is an elusive species with potential as a model species

Western European hedgehogs (*Erinaceus europaeus*) (hereafter hedgehogs) are a small, nocturnal and thus elusive mammal. With a highly flexible body covered in spines, they roam undetected in many habitats. They are described as 'edge' specialists moving through mosaic-like habitats, foraging in open and densely vegetated habitats and nesting in the later (Reeve, 1994; Huijser, 2000; Huijser and Bergers, 2000). Being hibernators and generalist feeders focusing on macroinvertebrates, they are affected by the ongoing decline in natural mosaic-like habitats, the decline in arthropods and climate change (Hof and Bright, 2010; Hallmann *et al.*, 2017). These species characteristics, alongside other abiotic and biotic factors like predation avoidance, availability of food and habitats, seem to drive them closer to and into urban environments, where they are found in higher numbers and where their population decrease seems slower than in rural habitats (Baker and Harris, 2007; Hubert *et al.*, 2011; Hof and Bright, 2012; *The State of Britain's Hedgehogs 2015*, 2015; C. E. Pettett *et al.*, 2017; B. M. Williams *et al.*, 2018). With their small home ranges, short generation times and lack of a clear dispersal phase in their ontogeny they are an ideal model species for other small mammals in urban environments (body size < 1500 g) (Doncaster and Dickman, 1987; Baker and Harris, 2007).

Intensive hedgehog research is, compared to their high public popularity, relatively scarce. In Europe, the research focus over the last 100 years has shifted during the decades between countries and was mainly driven by individual researchers. In the early 30s research on hedgehogs started with Konrad Herter in Germany (English version Herter 1965) and was picked up by Burton in United Kindom (UK) (Burton, 1969), while ecological field studies on hedgehogs were initiated through Pat Morris in the late 60s (Morris, 1973). In the 80s, Nigel Reeve undertook the first successful tracking studies of hedgehogs (Reeve, 1981). At the same time, the body of literature on hedgehog research increased in the European mainland with Reichholf and Esser in Germany (Reichholf and Esser, 1981), Kristiansson in Sweden (Kristiansson, 1984) and Boitani in Italy (Boitani and Reggiani, 1984). First genetic studies in the field were conducted by Becher and Griffiths in the UK (Becher and Griffiths, 1997). Other studies continued this research by resolving the genetic phylogeography and possible hybridisation (Santucci, Emerson and Hewitt, 1998; Seddon et al., 2001; Bolfíková and Hulva, 2012; Bolfíková et al., 2017). More recent studies resolved the origin and colonization of hedgehogs on islands on which they had been released (Bolfíková et al., 2013; Iannucci et al., 2018). Here, the hedgehog classified as an invasive species on island North and South island (New Zealand), Pinosa (Italy) and the Uists (Scotland), shows how the generalist traits described

earlier and its adjustment potential can be seen as a pre-adapted trait to novel habitats (Jackson and Green, 2000; Jackson, 2007; Bolfíková *et al.*, 2013). Nowadays, the European Hedgehog Research Group is active all over Europe and is beginning to incorporate other non-European hedgehog species as well, making the initiation of projects and future collaborations possible.

Recent studies, despite the broad geographical distribution, attempt to identify the causes for the ongoing decline of hedgehogs. Only the UK and the Netherlands have sufficient population data from countrywide and local surveys to scientifically identify a decreases in occupancy and declining abundance in their hedgehog population (Huijser and Bergers, 2000; Hof, 2009; Roos, Johnston and Noble, 2012; Johnson, 2015; Johnson *et al.*, 2015; Poel *et al.*, 2015; Hof and Bright, 2016; Carly E. Pettett *et al.*, 2017; Wilson and Wembridge, 2018). Following the numbers from the UK describing a serious decline in the hedgehog population, other countries slowly can add information to the bigger picture in Europe with local projects and support a general decline of hedgehogs e.g. in Denmark (Krange, 2015) and Germany (Müller, 2018).

While the ecology and needs of hedgehogs have been investigated in rural and semi-urban habitats, not much is known how cities, especially big cities, affect the ecology, genetics and behaviour of hedgehogs (Hubert *et al.*, 2011; Johnson *et al.*, 2015; Pettett, 2016; B. Williams *et al.*, 2018). More specifically, we lack information about the genetic diversity of hedgehogs in a highly urbanized and thus fragmented habitat. How do hedgehogs move and react in this highly changed and constantly changing environment? How do specific anthropogenic stressors affect hedgehogs? Moreover, how do individuals react to the extensive disturbance caused by transient events where a number of stressors add up?

Objectives & purpose of study

In order to address these questions, we needed to select 'state of the art' methods and establish a suitable study site. The chosen study site of Berlin is one of the biggest and yet one of the greenest cities in Europe, harbouring a variety of wildlife in and between its many green spaces (Kabisch *et al.*, 2016). Konrad Herter originally caught some of the hedgehogs for his studies back in the 1930s in Berlin. In 2013 the research project 'Igel in Berlin' was initiated. The aim of this study within the project is to investigate (i) whether the hedgehog population of Berlin is genetically affected by landscape fragmentation through urbanization. We aim to improve the methods for (ii) genetical and (iii) ecological studies, by ensuring high welfare standards. By utilizing the novel and improved method, we tried to (iv) understand the coping strategies of hedgehogs in urban parks under different highly changed circumstances and under natural

conditions. We used the novel approach of analysing hedgehog behaviour during a music festival, in order to (v) understand individual differences in coping strategies of hedgehogs.

Structure of this thesis

This thesis consists of five chapters, each presented as a manuscript on its own. The first part focuses on the population genetics of hedgehogs in two ways. Firstly, in Chapter 1 we report on our investigation of the genetic structure of hedgehogs in and around Berlin to reveal higher genetic connectivity between hedgehogs across the city than expected. In Chapter 2 in cooperation with colleagues from Vienna, Austria (University of Natural Resources and Life Sciences (BOKU)) we improved current genetic methods to sequence and analyse DNA samples for two hedgehog species in Europe; thus enabling higher resolution in future studies. Chapter 3 of the thesis reports our development of a new and improved method for the purpose of investigating individual behaviour of hedgehogs. By refining a backpack design, we made it possible to attach and reattach small lightweight devices like GPS, acceleration and other data loggers. In the final two chapters, we investigated the ecological consequences of human anthropogenic stressors on hedgehogs by utilizing proxies of movement, activity and nesting behaviour. Chapter 4 describes how we examined the effect on hedgehogs of a music festival and fragmentation in two resident populations of urban parks. While in the last Chapter (5) we report on our investigation into the individual coping strategies of hedgehogs during the previously mentioned music festival.

Manuscripts and Author contribution

1. Barthel L, Wehner D, Schmidt A, Berger A, Hofer H, Fickel J Unexpected gene-flow in urban environments: the example of the European hedgehog *submitted to PeerJ*

   Contributions: Conceived and designed the experiments: LB JF. Performed the experiments: LB DW AS. Analysed the data: LB JF Contributed reagents/ materials/analysis tools: AB HH JF. Wrote the first draft of the manuscript: LB Read and improved the manuscript: AB HH JF. All authors contributed critically to the drafts and gave final approval for publication.

2. Curto M, Winter S, Seiter A, Schmid L, Scheicher K, Barthel L, Plass J, Meimberg H Application of an SSR-GBS marker system on an investigation of European Hedgehog species and their hybrid zone dynamics. Ecol Evol. 2019;9:2814–2832. https://doi.org/10.1002/ece3.4960

   Contributions: The Experiment was planned and designed by HM and MC. MC and SW wrote the bioinformatics pipeline with contributions of KS. Genotypes were analysed by MC, HM, LS and AS. LS and AS part of the dataset as in the context of their master theses. LB and JP provided and organized the samples. HM and MC led the writing of the manuscript with contributions of all authors.

3. Barthel L, Hofer H, Berger A. An easy, flexible solution to attach devices to hedgehogs (*Erinaceus europaeus*) enables long-term high-resolution studies. Ecol Evol. 2019;9:672–679. https://doi.org/10.1002/ece3.4794

   Conceived and designed the experiments: LB AB HH. Performed the experiments: LB. Analysed the data: LB Contributed reagents/ materials/analysis tools: AB HH. Wrote the first draft of the manuscript: LB Read and improved the manuscript: AB HH. All authors contributed critically to the drafts and gave final approval for publication.

4. Barthel L, Gras P, Berger A, Rast W, Hofer H Distinguishing spatial from temporal effects in disturbance biology: Hedgehogs in the urban matrix of habitat fragmentation and noise pollution *draft*

   Conceived and designed the experiments: LB AB HH. Performed the experiments: LB. Analysed the data: LB PG WR Contributed reagents/ materials/analysis tools: AB HH. Wrote the first draft of the manuscript: LB Read and improved the manuscript: HH. All authors contributed critically to the drafts and gave final approval for publication.

5. Rast W, Barthel L, Berger A Music festival makes hedgehogs move: How individuals cope behaviorally in response to human-induced stressors *submitted to Animal*

   A.B. and L.B. conceived the ideas and designed methodology; L.B. collected the data; W.R., L.B. and A.B. analysed the data; W.R. and A.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

1 Unexpected gene-flow in urban environments: the example of the European hedgehog

Submitted to PeerJ, March 2019

Leon M.F. Barthel [1,2], Dana Wehner[3], Anke Schmidt[4], Anne Berger[1,2], Heribert Hofer[2,3,5,6], Jörns Fickel[4,7]

[1]Department of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Straße 17, 10315 Berlin, Germany
[2] Berlin Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany
[3] Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany
[4] Department of Evolutionary Genetics, Leibniz Institute for Zoo and Wildlife Research (IZW)
[5] Department of Ecological Dynamics, Leibniz Institute for Zoo and Wildlife Research (IZW)
[6] Department of Veterinary Medicine, Freie Universität Berlin, Oertzenweg 19b, 14195 Berlin, Germany
[7] Institute for Biochemistry & Biology, University of Potsdam, 14476 Potsdam-Golm, Germany
Corresponding Author:
Leon M.F. Barthel /Jörns Fickel

1.1 Abstract

Urban environments are characterized by high structural diversity. This includes strong habitat fragmentation and many landscape types, which provide a rich matrix of novel, mosaic-like and sometimes fast changing habitat patches. Such structural diversity is likely to influence the genetic structure of urban wildlife, especially in species with small home range sizes and limited ability to surmount barriers. The presence of such barriers will cause genetic isolation of populations, whereas structures facilitating connectivity across different urban landscapes will allow gene-flow and thus support the persistence of sustainable populations. Here we use the European hedgehog (*Erinaceus europaeus*), a small mammal with limited mobility, as a model species to study if the structural matrix of the urban environment has an influence on genetic population structure in the city of Berlin (Germany). Using 10 established microsatellites, we genotyped 143 hedgehogs from numerous sites throughout Berlin. The inclusion of all individuals into the cluster analysis yielded three genetic clusters, potentially reflecting spatial associations of kin (larger family groups). To examine the potential bias from the cluster analysis potentially caused by closely related individuals, we determined all pairwise relationships and excluded close relatives before repeating the cluster analysis. For this data subset (N = 65) both clustering algorithms (Structure, Baps) applied indicated the presence of a single genetic cluster. These results suggest that the high proportion of green patches in the city of Berlin provides numerous stepping stone habitats potentially linking local subpopulations, although we cannot exclude translocation of individuals by hedgehog rescue facilities, which would facilitate gene flow as well. We therefore propose that management activities such as releases by animal rescue centres should become a part of the data collection of population genetic studies.

Keywords: urban wildlife, conservation, genetic population structure, habitat fragmentation, wildlife genetics, Habitat connectivity, fragmentation, Urbanization

1.2 Introduction

Urbanization involves some of the most rapid and intense human-induced transformation processes of the formerly existing landscape. Structures such as impervious surfaces, roads and buildings have fragmented the environment for many species. Smaller patches of the former landscape are now separated by semi-penetrable or impenetrable barriers. In order to gain access to adequate resources, animals living on such patches often have to cross these barriers to move from one patch to another. Some wildlife species are able to surmount barriers and can cope with urban conditions (urban utilizers, urban dwellers; Fischer et al. 2015) and the close proximity to people (e.g., wild boar *Sus scrofa*; Stillfried et al. 2017), whereas others cannot (e.g., great bustard *Otis tarda*; Pitra et al. 2002). Thus, for behaviourally flexible wildlife species urban habitats may provide a novel living environment with the opportunity to exploit novel resources (Johnson & Munshi-South 2017; Stillfried et al. 2017; Maclagan et al. 2018).

Geographic separation of populations by barriers reduces gene-flow among them and thus increases genetic differentiation among populations. It also decreases the genetic variation within populations both by genetic drift and by reducing the availability of genetically different breeding partners, thereby increasing the risk of inbreeding and subsequent inbreeding depression as well as of higher infection rates and thus elevated mortality (Belasen et al. 2019). Thus, a consequence of habitat fragmentation may be local population extinction (Wilcox & Murphy 1985; Munshi-South & Nagy 2014; Lourenço et al. 2017; Martins et al. 2017).

However, urban landscapes may be intermingled with large green patches such as parks, residential gardens, cemeteries, but also with currently unused former industrial sites and other habitats that still can provide a living space for wildlife species. These patches may serve as stepping stone habitats, allowing gene flow between otherwise separated local populations (Kimura & Weiss, 1964). Whether gene flow occurs depends on the mobility and dispersal capacity of each species in relation to the distances between suitable habitat patches and their distribution within the urban matrix. Thus, we expect species with high mobility and high dispersal capacity to be less affected by a strongly structured urban landscape than species with small home ranges and limited dispersal capacity. For the latter we therefore expect a fragmented urban landscape to promote genetic isolation of clusters of individuals, causing a highly structured meta-population (Opdam 1991; Andrén 1994; Taylor et. al 2011; Braaker et al. 2017).

The purpose of this study is to test these expectations by genotyping European hedgehogs (*Erinaceus europaeus*) across the highly fragmented urban matrix of the city of Berlin. Although hedgehogs are widely distributed across Europe (Amori 2016), we used this species as a model species because of its limited dispersal capacity and its relatively small home range (Doncaster & Dickman 1987; Baker & Harris 2007). The size of the latter may range from 0.8 ha (England, Dowding et al. 2010), over 10 to 40 ha (England, Morris 1988) up to 98 ha (Finland, Rautio et al. 2013). While female hedgehogs mostly stay within their habitat patches, male hedgehogs occasionally cover distances of up to 7 km per night (Zingg 1994). Because the European hedgehog can use the urban matrix and cope with its structural characteristics (Doncaster & Dickman 1987; Braaker et al. 2014), population densities in urban areas can be higher than in rural habitats (Hubert et al. 2011; Hof et al. 2012). Despite their broad geographical distribution and their ability to utilize urban matrices, hedgehog populations have been declining in size and numbers across Europe (Huijser & Bergers 2000; Krange 2015; Hof & Bright 2016; Müller 2018). Understanding the long-term consequences of progressive spatial fragmentation by urbanization on hedgehog genetic population structure might become increasingly important for developing conservation strategies for this species (Doncaster & Dickman 1987; Hof & Bright 2009; Beninde et al. 2015).

1.3 Materials & Methods

Sample collection and sites: Over a period of five years (2013-2017), we collected mouth mucosal cells of free-ranging European hedgehogs (N = 250) using cotton swabs (FLOQSwabs, COPAN, Brescia, Italy and Forensic Swabs, Sarstedt, Nümbrecht, Germany) in the city of Berlin and its suburbs (~1050 km²). Sampling was carried out during torchlight transect walks between 10 p.m. and 4 a.m. in different public parks, cemeteries and green areas in Berlin, whereby in 2016 and in 2017 the main focus was on two large parks: a) the 'Treptower Park', a 20 ha open public park in south-central Berlin and b) the ~160 ha large 'Tierpark Berlin' (one of Berlin's two zoological gardens). For each individual, we recorded the GPS coordinates of its location. Additional samples (N = 56) were provided by animal rescue facilities and local veterinary surgeries in Berlin (Figure 1.1, Supplementary Table 1.1). For these samples approximate locations were provided by staff members. Coordinates, which may have had an error margin of a few hundred meters were recorded using online maps. We also asked staff working at these facilities whether they had implemented particular rules on how release sites were chosen after the rehabilitation of hedgehogs. All procedures in this study involving animals were performed in accordance with the ethical standards of the institution (IZW permit 2016-02-01) and German federal law (permission numbers Reg0115/15 and G0104/14).

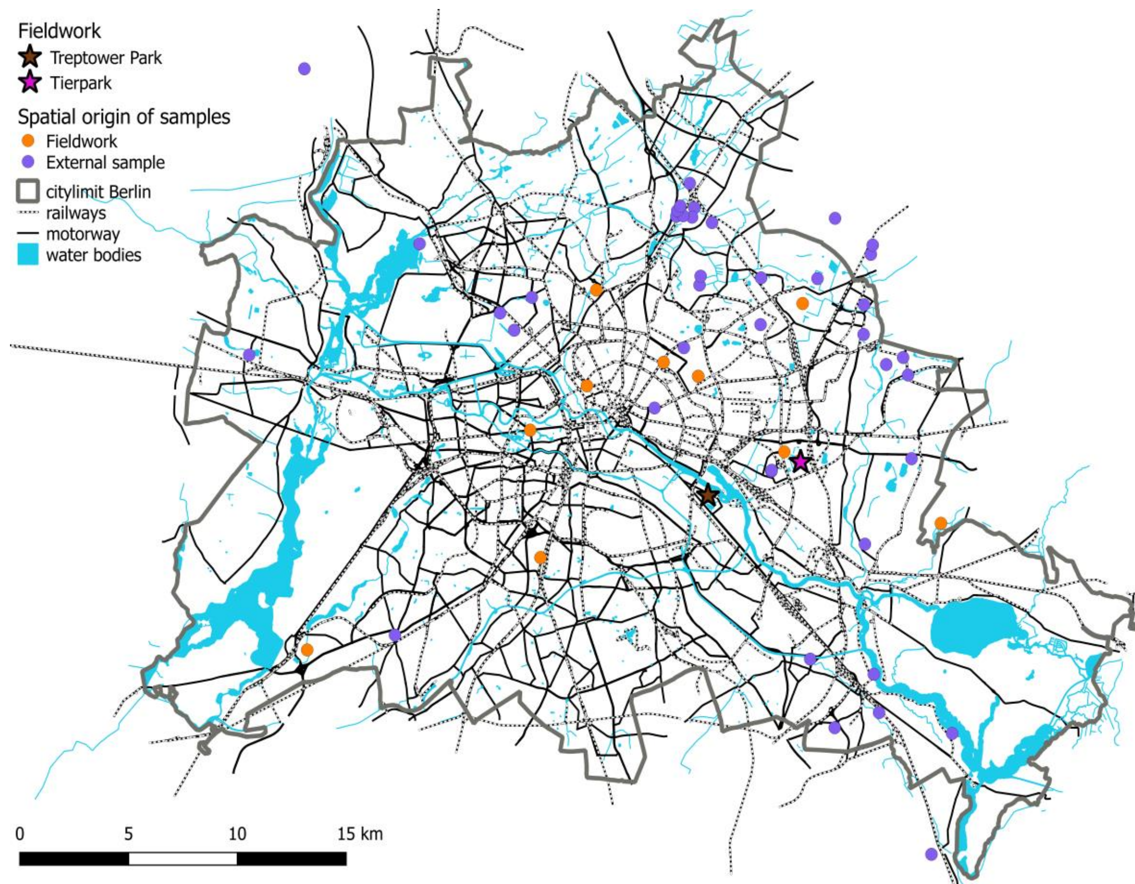


Figure 1.1: Map of Berlin and its surroundings and showing the locations from 139 out of 143 samples (four samples not shown because outside of the map)

<u>DNA extraction and analysis of microsatellite loci</u>: DNA was extracted from all 306 samples using the DNeasy kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, with a final DNA-elution in 80 µl distilled water (sterile). DNA concentrations were measured spectrophotometrically using a NanoDrop1000 (PeqLab GmbH, Erlangen, Germany). Individuals were genotyped at 10 microsatellite loci using a panel of nine loci from a previous landscape genetics study (Bolfíková & Hulva 2012), with locus EEU1 added. The panel consisted of the following loci: EEU1, EEU2, EEU3, EEU4, EEU5 and EEU6 (Becher & Griffiths 1997), EEU12H, EEU37H, EEU43H, and EEU54H (Henderson et al. 2000). One primer per pair was 5`- labelled with a fluorescent dye (6-FAM or HEX). To save time and costs, we prepared (after optimization) four primer master-mixes (Mix-A to Mix-D, 50 µl each). Mix-A contained the primers for loci EEU1, EEU2, and EEU54H (all 1 µM), Mix-B consisted of primers for loci EEU6 (1 µM) and EEU12H (2 µM), Mix-C of primers for loci EEU3 (1µM) and EEU37H (2 µM), and Mix-D included the primers for loci EEU4 (4 µM) and EEU5 (2 µM). Primer pair EEU43H (3 µM) was run separately. The genotyping PCR mixture (10 µl) consisted of 5µl 2× Type-itTM multiplex mix (Qiagen, Hilden, Germany), 1 µl primer mix, 3 µl H2O and 1 µl DNA (50-120 ng). Cycling conditions were equal for all four master-mixes and locus

EEU43H and were performed as touchdown-PCR: 95 °C 5 min, 4× {94 °C 30 s, 63 °C down to 57 °C in 2 °C increments of 90 s each, 72 °C 30 s}, 31× {94 °C 30 s, 55 °C 90 s, 72 °C 30 s}, 60 °C 30 min final elongation. Amplification products were analysed by capillary electrophoresis on an A3130xl automated sequencer (Thermo Fisher, Waltham, USA) using POP7 and sized by comparison to a Genescan™ 500 ROX™ Size Standard (ABI) using the software Genemapper v.3.7 following the manufacturer's instructions. To avoid misleading results by allelic dropouts and false alleles, we applied a maximum likelihood approach (Miller et al. 2002) and genotyped each sample twice (in duplicates). We did not allow for any allele mismatch between duplicates. If there was a mismatch, the sample was removed and genotyped again in duplicates from freshly extracted DNA. Genotypes were only scored if no mismatch was detected; otherwise the sample was excluded from further analysis. We also excluded all individuals for which more than one locus had missing data.

Data analysis: We calculated observed (HO) and expected heterozygosities (HE), number of alleles (NA), as well as potential deviations from Hardy-Weinberg equilibrium (HWE) using the program Cervus v.3.0.7 (Guo & Thompson 1992; Marshall et al. 1998; Kalinowski et al. 2007). We also used Cervus to search for matching genotypes across all samples. Tests for the presence of genotypic disequilibria among loci were performed using the software package Arlequin v.3.5.2.2 (Excoffier et al. 2005; Excoffier & Lischer 2010). The significance level α was Bonferroni-corrected and set at 0.001 (0.05: 45 pairwise comparisons). Potential presence of null alleles was assessed using Micro-Checker v.2.2.3 (Van Oosterhout et al. 2004).

Although hedgehogs are solitary animals, their limited dispersal capacity (compared to larger mammals) may cause a population genetic structure by which closely related individuals may be living in closer proximity to each other than to more unrelated individuals. Because some clustering algorithms are affected by such associations of kin (Rodríguez-Ramilo & Wang 2012), we determined pairwise relatedness (r; Queller & Goodnight 1989) among all samples using the software package Coancestry v.1.0.1.9 (Wang 2011). Pairs with $r > 0.5$ were marked and subsequent cluster analysis (see below) was performed with and without these pairs (Supplementary Figure 1.2).

The possible presence of genotypic clusters was evaluated both for the subset of only unrelated individuals (N = 65) and for the whole data set (N = 143). Here for this purpose, we used two software packages with a Bayesian clustering approach: Structure v.2.3.4 (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009) and Baps v.6.0 (Corander et al. 2008, Cheng et al. 2013). As priors for Structure, we applied the admixture model in conjunction with the correlated allele

frequency model, because it is better suited to detect a subtle population structure, although this makes it more likely to overestimate the number of clusters K (Falush et al. 2003). The model was applied to K-values ranging from K = 1 to 8. The required allele frequency distribution parameter λ was estimated per run. To determine both appropriate burn-in and Markov chain lengths for parameter estimates of allele frequencies and membership coefficients per genotype in each genotypic cluster (Q), we set K = 1 and watched for the likelihoods to converge under various burn-in and run lengths. The final burn-in length was set at 20 000 iterations and Markov chains were run with a length of 200.000 iterations each. Each K was independently assessed 10 times to verify the consistency of estimates across runs. The most likely K was determined using both the log likelihood values (as ΔK cannot be applied if K = 1) and by following the ΔK method (Evanno et al. 2005) using Structure Harvester (Earl & von Holdt 2012). For Baps the K prior ranged from 2 to 8 (as Baps cannot detect K = 1), whereby each K was independently assessed 10 times. In addition, we used a location prior by providing the GPS coordinates of each sample's origin. We applied the algorithm to both 'admixture' and 'no admixture'.

Assignment: The threshold for the Q-value above which an individual will be assigned to a cluster is of importance because if the threshold is too high it may underestimate a structure that in reality exists, whereas a threshold which is too low will overemphasize a structure that in reality is not as pronounced as assumed. Here we chose a relatively conservative value of Q ≥ 0.85 as the threshold for the assignment of individuals, thus allowing for some gene flow to have occurred among the inferred ancestral populations. Genetic distances between clusters of assigned individuals as well as the number of migrants (Nm) among clusters were estimated using Arlequin. Input files for the different programs were generated using the software Create (Coombs et al. 2008). Data are presented as means ± standard deviations (SD) unless otherwise stated.

1.4 Results

Genotyping: From the original dataset of 306 hedgehog samples, data from 156 individuals had to be excluded - 154 individuals were excluded because genotyping of their samples failed at more than one locus and two individuals were removed because the alleles of their duplicate sample genotypes did not match at all loci. In total, 150 (49%) were successfully genotyped at all ten loci. Out of these genotypic profiles, one profile occurred three times and five others twice, leaving 143 unique genotypes (Supplementary Table 1.2). The number of alleles per locus (N_A) ranged from four (locus EEU12H) to 16 (EEU37H), with a mean of 10.9 ± 4.1 (Table 1.1). H_O across all 143 unique genotypes ranged from 0.350 at locus EEU6 to 0.754 at locus EEU3, with

a mean of $H_O = 0.621 \pm 0.133$ (Table 1.1). Across all loci and individuals one locus (EEH37H) deviated significantly from HWE (Table 1.1). Although several loci indicated the potential presence of null alleles, the probability was generally very low.

Table 1.1: Indices of ten microsatellite loci across the 143 unique genotypes

Locus	N_{typed}	N_A	Allele size range (bp)	H_O	H_E	HWE	f_{Null}
EEU1	143	8	129 - 143	0.671	0.773	+	<u>0.062</u>
EEU2	141	13	257 - 281	0.752	0.863	+	<u>0.064</u>
EEU3	142	15	131 - 181	0.754	0.868	+	<u>0.064</u>
EEU4	143	14	144 - 170	0.699	0.785	+	<u>0.052</u>
EEU5	143	13	107 - 139	0.678	0.711	+	<u>0.011</u>
EEU6	143	6	145 - 159	0.350	0.331	+	-0.049
EEU12H	143	4	91 - 97	0.497	0.615	+	<u>0.098</u>
EEU37H	142	16	236 - 280	0.676	0.839	-	<u>0.095</u>
EEU43H	143	12	146 - 172	0.657	0.730	+	<u>0.047</u>
EEU54H	142	8	276 - 296	0.479	0.551	+	<u>0.067</u>
Mean	142.5	10.9		0.621	0.707		
SD	0.71	4.09		0.133	0.167		

N_{typed}: number of individuals successfully genotyped at that locus, N_A: number of alleles per locus, bp.: base pairs, H_O: observed heterozygosity, H_E: heterozygosity expected under HWE, HWE: Hardy-Weinberg equilibrium. (+): locus was at HWE, (-): locus deviated from HWE, f_{Null}: probability for the presence of null-alleles (underlined values indicate the potential presence of null-alleles), SD: standard deviation.

Pairwise relatedness analysis revealed numerous pairs of individuals with a high relatedness index ($r \geq 0.5$). Removal of these related individuals reduced the data set to 65 unrelated hedgehogs. Presence of linkage disequilibria (LD) among the ten loci was tested both for 65 hedgehogs (unrelated individuals) and for 143 hedgehogs (all individuals). Among the 65 hedgehogs one out of 45 pairwise comparisons among the ten loci and among the 143 hedgehogs 12 pairwise comparisons showed LD, although all loci had previously been declared to be independently inherited (Becher & Griffiths 1998; Henderson et al. 2000; the latter also included the loci from Becher & Griffiths 1998, Bolfíková & Hulva 2012). In our study, hedgehogs were sampled over a very large area, therefore potentially violating the assumption of an unstructured population, as expected for a small mammal in a highly fragmented landscape. The deviation from HWE at locus EEU37H and the linkage disequilibria may thus have been the result of the Wahlund effect

(Wahlund 1928). We therefore searched for an underlying population structure, first among the unrelated individuals and then among all individuals.

When analyzing the 65 unrelated individuals (Figure 1.2), all individuals were assigned to a single cluster (mean LnP(K) = -2268.81 ± 0.481; results from Structure). When analyzing all 143 individuals (related + unrelated), both clustering algorithms (Structure, Baps) indicated the presence of three to four genotypic clusters (Table 1.2). The ΔK estimate (Structure Harvester) favoured three clusters over four (ΔK for K = 3 was 52.25; ΔK for K = 4 was 51.4), whereas Baps favoured the presence of four clusters, with the fourth cluster being represented by two individuals (sampled at the same location). The likelihood for the number of genotypic clusters (K) to reflect the true number of ancestral populations had the following values (derived from Baps): for K = 3: 0.00136, K = 4: 0.98883, and for K = 5: 0.0098). Using a value of $Q \geq 0.85$ (Structure), 74 out of 143 genotypes (~52%) were assigned to either one of three genotypic clusters: cluster 1 with 29 genotypes, cluster 2 with 14 genotypes (all individuals but one were from 'Tierpark'), and cluster 3 with 31 genotypes (all but one from 'Treptower Park'). The 69 remaining genotypes were admixed, with admixture occurring across all clusters (Table 1.2, Supplementary Figure 1.1). Each cluster was at HWE. Observed (HO) and expected heterozygosities (HE) were HO = 0.623 and HE = 0.685 for cluster 1 (N = 29), for cluster 2 (N = 14) they were HO = 0.557 and HE = 0.524, and for cluster 3 (N = 31) they were HO = 0.578 and HE = 0.651. Pairwise genetic distances (FST) among all clusters were significant ($p < 0.05$) with FST = 0.169 between clusters 1 and 2, FST = 0.11 between clusters 1 and 3, and FST = 0.192 between clusters 2 and 3.

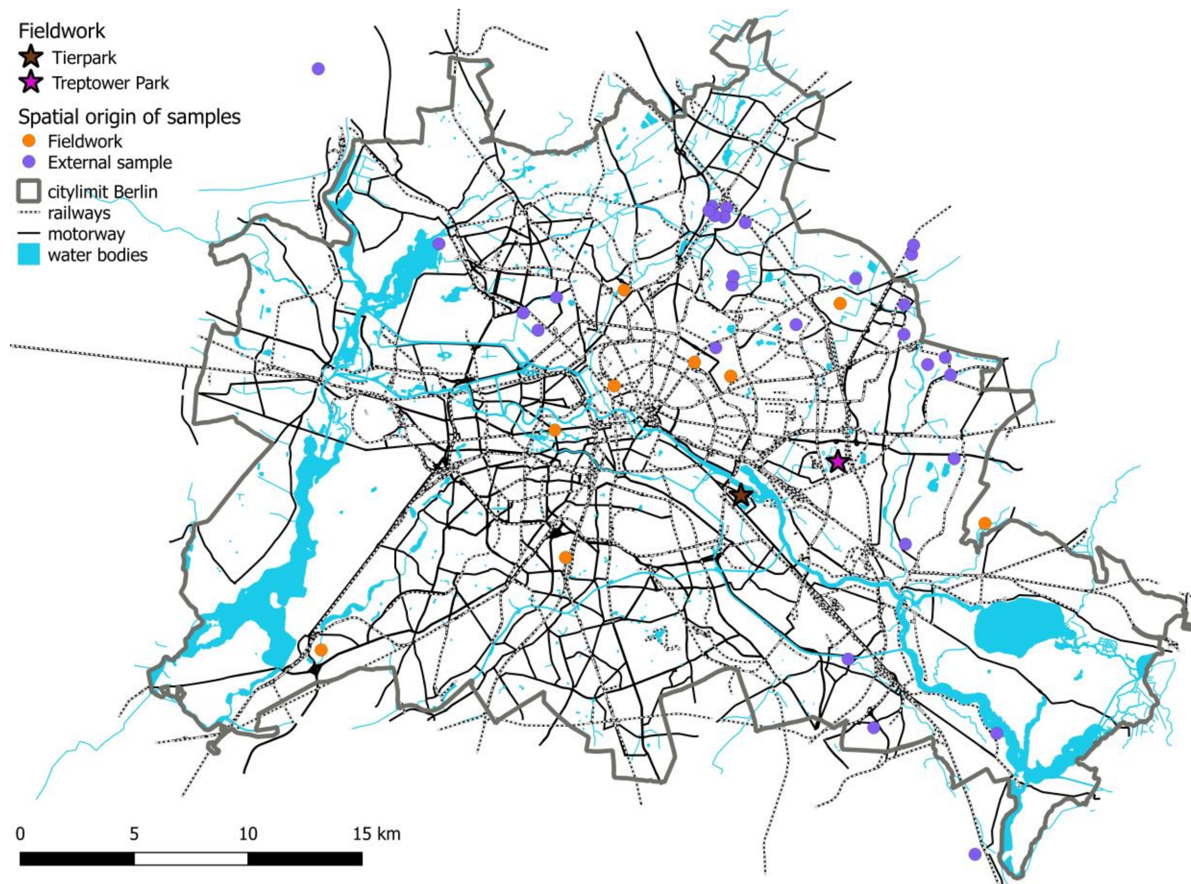


Figure 1.2: Map of Berlin and its surroundings and showing the locations from 64 out of 65 unrelated samples (one sample not shown because it was located 70 km north of Berlin), later by Structure identified as one cluster

The number of migrants (Nm) per generation also differed among the three clusters. It was Nm = 1.22 between clusters 1 (wide-spread) and 2 ('Tierpark'), Nm = 2.02 between clusters 1 and 3 ('Treptower Park') and Nm = 1.05 between clusters 2 and 3. Applying the Baps clustering algorithm led to results very similar to the ones obtained from the Structure analysis, except for the introduction of a fourth cluster (2 individuals only) and an increase in the number of hedgehogs assigned to any cluster (Table 1.2). This increase in the number of individuals assigned to a cluster was particularly pronounced in cluster 1, into which Structure had only assigned 29 hedgehogs, whereas the Baps algorithm assigned three times as many individuals to that cluster (N = 87). Following the Baps assignment, hedgehogs from cluster 1 were also present in the 'Tierpark' and the 'Treptower Park'.

Release of hedgehogs after rehabilitation: Although rescue facility's had no particular rules regarding the selection of release sites for rehabilitated hedgehogs, general policy was to release hedgehogs into favourable habitats, independent of their point of geographic origin. This lead to the release of hedgehogs at distances far from the facilities, in some cases at distances of >100 km.

1.5 Discussion

Considering only unrelated individuals, hedgehogs were assigned to a single cluster whose members were spread across the city (Figure 1.2). Such lack of genetic population structure despite the presence of many potential barriers was surprising, and is in contrast to results from urban hedgehogs in the City of Zürich (Switzerland), where a strong differentiation had been observed in a study on 149 hedgehogs in an area of ~100 km² (Braaker et al. 2017). There, despite the much smaller spatial scale, three genotypic clusters had been inferred. The Zurich hedgehog clusters were well delineated by a major inner-city transportation axis as an anthropogenic barrier and two rivers as natural barriers (Braaker et al. 2017). The authors concluded that urban green areas were the most suitable habitat type to facilitate gene flow, whereas all other land cover types were more likely to impede gene flow (Braaker et al. 2017).

The Zurich study differed from ours in several aspects: Their threshold for assigning individuals to a genetic cluster was considerably lower ($Q \geq 0.65$ instead of $Q \geq 0.85$), and they did not consider the potential effect of association of kin on genetic population structure. In our study, unrelated individuals did not demonstrate any obvious population genetic structure, although the city of Berlin is much larger than Zurich and even more divided by several highways and large rivers or canals.

The inclusion of all individuals indicated the presence of at least three genotypic clusters ($Q \geq 0.85$), two of which were spatially well delineated (clusters 2 and 3, STRUCTURE, the 'Tierpark' and the 'Treptower Park'). As this population genetic structure only appeared if related individuals were included into the cluster analysis, we suggest the differentiation detected here to be a reflection of an underlying kinship network of 'family clans' rather than to be a reflection of allele frequencies of three ancestral populations. Such a 'family clan' structure would also explain the local concentration of cluster 2 individuals in the 'Tierpark' and of cluster 3 individuals in the 'Treptower Park'. Although the 'Tierpark' is a large park-like area (~160 ha) that was preserved after WW II and established as a zoological garden in 1954, it is fully fenced and surrounded by big streets in the north and west and railway tracks in the east and south. Thus, gene flow between hedgehogs from the 'Tierpark' and the surrounding areas is clearly restricted, explaining the confinement from hedgehogs of cluster 2 to the 'Tierpark'. This is also evidenced by the significant pairwise F_{ST} values, which were the highest between clusters 2 and 3 ($F_{ST} = 0.192$) and clusters 2 and 1 ($F_{ST} = 0.169$). Interestingly, the hedgehogs inhabiting the 'Treptower Park' (cluster 3) are only strongly differentiated from the ones living in the 'Tierpark' ($F_{ST} = 0.192$, lowest migration rate with $N_m = 1.05$), but not from the wider-spread cluster 1 ($F_{ST} = 0.11$,

highest migration rate with $N_m = 2.02$). The main difference to the location 'Tierpark' is that the 'Treptower Park' is not fenced in and always accessible. However, it is bordered on one side by the river Spree and on its three other sides by heavy-traffic roads. An additional heavy-traffic road is crossing the park longitudinally. Yet these barriers appear still to be more penetrable for hedgehogs than those in the 'Tierpark'. This leads to a question; why have these family-clans become so large? We think the reason for that is the low landscape resistance within the parks, whereas at the borders of the parks landscape resistance increases drastically (cf. Braaker et al. 2017).

Expected heterozygosity (H_E) for individuals of the most wide-spread cluster (cluster 1: H_E = 0.685; N = 29) was similar and even higher than the value of $H_E = 0.68$ measured in a country-wide study in the Czech Republic (average sample site distances >450 km; Bolfíková & Hulva 2012), indicating that in the case of Berlin, the urban environment might not lead to a reduction of genetic variability in hedgehogs. Because individuals from cluster 2 and 3 of our study were confined to single parks, either to the 'Tierpark' or to the 'Treptower Park', we expected to detect low observed (H_O) and expected heterozygosities (H_E) there. Even though the values were indeed lower than in the widespread cluster 1, they were only lower by a small margin (cluster 2: $H_O = 0.557$/ $H_E = 0.524$; cluster 3: $H_O = 0.578$/ $H_E = 0.651$). These values were similar to those measured in free-ranging hedgehog populations from New Zealand affected by a founder effect (H_O = 0.42-0.52/ H_E = 0.51-0.57; Bolfíková et al. 2013).

In contrast to many other mammalian species, Hedgehogs seem to lack a clear dispersal phase (Morris & Reeve 2008). They rarely cover distances larger than 4 km (Morris & Reeve 2008) and are restricted in their movements by roads and other barrier-like structural elements (Doncaster et al. 2001; Rondinini & Doncaster 2002). Thus, the emergence of a genetic population structure due to restricted gene flow as seen in Zurich appears to be inevitable. Yet hedgehogs in Berlin did not differentiate into such a clear population genetic structure, although the city of Berlin is much larger (875.94 km^2) than Zurich. We thus expected dispersal to be even less likely and therefore a genetic population structure to be even more pronounced and clearly delineated by space. This was not the case. Our results and observations would be compatible with the idea that all Berlin hedgehogs derived from a single ancestral population.

But because our results provide only a temporal snapshot, we do not know whether the spatial discrimination of clusters 2 ('Tierpark') and 3 (Treptower Park') is the beginning of a process leading either to population differentiation or to complete admixture, or whether it may represent a stable genotypic equilibrium.

Although we currently do not have detailed knowledge about the ancestry of hedgehogs in Berlin, it is well known that hedgehogs have lived for centuries in Berlin and have experienced Berlin's increasing urbanization throughout this period (Herter 1933). This raises the question as to what could be the reasons for the lack of a clear, spatially derived population genetic structure in a species that is considered to be substantially constrained by physical urban structures such as waterways, motorways, railways, and built-up areas (Doncaster et al. 2001; Baker & Harris 2007; Morris & Reeve 2008), structures that characterize Berlin?

We argue that the main reason for our finding is the large proportion of green areas in Berlin. The City of Berlin (87.594 ha) is covered by 15.752 ha of forests (18 %) and 10.885 ha of public green patches (12.4 %), such as cemeteries, parks and gardens (SenUVK, 2018, Stillfried et al. 2017). These areas provide a connective web of suitable habitats within the urban matrix, improving the opportunities for hedgehogs to maintain some amount of gene flow across the city. However, we cannot exclude the effect of other factors on admixture. Given home ranges of 0.77 – 2.78 ha in residential areas (Dowding et al. 2010), the distances to be covered to establish gene flow between 'family clan' clusters are quite large for a short-legged ground-dwelling species, although numerous small and larger green areas can be stepping stones to link distant parts of the city. We therefore assume that admixture will have been enhanced by animals released by hedgehog rescue facilities. These events are at present not fully quantified, but our interviews with personnel from rescue facilities confirmed that they are a regular occurrence. Such rescue related translocations have also been observed in other studies (e.g. Molony et al. 2006 or Braaker et al. 2017).

1.6 Conclusions

We hypothesized that urban hedgehog, a species with relatively low mobility and low dispersal capacity, will be highly influenced by fragmented urban landscapes leading to genetic isolation of populations and thus a highly structured meta-population. Yet the hedgehog population in the city of Berlin is not genetically structured if only unrelated individuals are being taken into account. A population genetic structure becomes only visible if related individuals are also included in the analysis. These 'family clan'-clusters are likely realized naturally across the numerous green patches Gene flow between of Berlin's urban matrix although anthropogenic translocations cannot be excluded. To maintain the currently existing genetic diversity in Berlin's hedgehog population, we suggest its repeated monitoring by census measures and population-wide genetic analysis to determine if current clusters ('family clans') are at risk of becoming isolated.

Table 1.2: Results of both clustering algorithms (Structure, Baps) and their assignment for each sample

internal ID	locality	STRUCTURE Q-value for cluster 1	STRUCTURE Q-value for cluster 2	STRUCTURE Q-value for cluster 3	Assignment by STRUCTURE Q ≥ 0.85	Assignment by BAPS (location prior)
147	Tiergarten, Berlin	0,87559	0,10548	0,01895	1	4
167	Tiergarten, Berlin	0,88068	0,10901	0,01031	1	4
176	Eisenhuettenstadt	0,85406	0,11966	0,02629	1	1
161	Hans-Baluschek-Park,	0,86127	0,11218	0,02655	1	1
311	Tierpark, Berlin	0,87213	0,10962	0,01824	1	1
334	12623 Berlin	0,86935	0,11628	0,01438	1	1
335	12623 Berlin	0,85235	0,11496	0,03271	1	1
220	Friedenstr., Berlin Near Volkspark Friedrichshain	0,87103	0,11977	0,00921	1	1
175	Volkspark Prenzlauerberg, Berlin	0,86135	0,105	0,03368	1	1
199	Hellersdorf,Berlin	0,86134	0,12691	0,01175	1	1
159	Prenzlauerberg	0,85732	0,10238	0,04031	1	1
338	Park am Weidengrund	0,87595	0,11224	0,01182	1	1
117	Near graveyard	0,88408	0,10591	0,01	1	1
326	Zum Erlenbruch, 15344 Strausberg	0,86423	0,11255	0,02321	1	1
156	Buergerpark Pankow-Berlin	0,86845	0,11301	0,01854	1	1
341	Kleingartenanlage 750 Jahre Berlin, 13057 Berlin	0,85329	0,10368	0,04302	1	1
328	Warnem ▪ der Str. 18, 13059 Berlin	0,86432	0,11747	0,01821	1	1
329	Warnem ▪ der Str. 18, 13059 Berlin	0,86724	0,1151	0,01767	1	1
330	Warnem ▪ der Str. 18, 13059 Berlin	0,87485	0,1077	0,01746	1	1
337	KGA Maerchenland, 13089 Berlin	0,87728	0,10819	0,01456	1	1
231	Friedenstr. 8, 16356 Ahrensfelde	0,87273	0,10608	0,02116	1	1
257	Dietrichstr. 5, 16356 Ahrensfelde	0,87167	0,10645	0,0219	1	1
193	Jungbornstr., 13129 Berlin	0,85709	0,12218	0,02072	1	1
189	Strasse 7, 13129 Berlin	0,85836	0,11349	0,02814	1	1
194	Schwarzwaldstr./Ilsenstr., 13129 Berlin	0,85756	0,11789	0,02457	1	1

185	Gutenfelsstr. 14, 13129 Berlin	0,8736	0,11369	0,01271	1	1
187	Gutenfelsstr. 14, 13129 Berlin	0,8837	0,1066	0,0097	1	1
113	Choise-le-Roi-Str. 3, Berlin	0,88496	0,10623	0,00881	1	1
118	Vielitzsee Ortsteil Strubensee, 16835 green area	0,85436	0,13413	0,01153	1	1
179	Eisenhuettenstadt	0,84074	0,10226	0,05701	admixed	1
243	Zeuthen	0,6249	0,20766	0,16744	admixed	1
129	Rohrwallallee 10, 12527 Berlin	0,83142	0,10412	0,06446	admixed	1
120	Altglienike Feldweg	0,84766	0,10475	0,0476	admixed	1
137	Kablower Weg 89, 12526 Berlin	0,78369	0,0984	0,1179	admixed	1
138	Kablower Weg 89, 12526 Berlin	0,8267	0,1031	0,0702	admixed	1
125	Riesserseestr. 10, 12527 Berlin	0,84665	0,11413	0,03923	admixed	1
135	Korkedamm 73, 12524 Berlin	0,69588	0,08972	0,21436	admixed	1
127	Rehwiese, Gerkrathstraße 2 Park	0,72131	0,14963	0,12906	admixed	1
182	Zehlendorf, Berlin	0,40151	0,09898	0,49948	admixed	1
158	Hans-Baluschek-Park, 10829 Berlin	0,56664	0,07147	0,36189	admixed	1
169	Hans-Baluschek-Park, 10829 Berlin	0,75432	0,10163	0,14405	admixed	1
235	Glasberger Str. 43, 12555 Berlin	0,77728	0,10906	0,11368	admixed	1
110	Trainierbahn Hoppegarten	0,55419	0,42731	0,01849	admixed	1
A35_088	Treptower Park	0,66656	0,14738	0,18608	admixed	1
A4_317	Treptower Park	0,81876	0,16614	0,01512	admixed	1
A61_108	Treptower Park	0,67049	0,09899	0,23051	admixed	1
A68_108	Treptower Park	0,71856	0,17835	0,10308	admixed	1
126	Moldaustr. 30, 10319 Berlin near Tierpark	0,68485	0,2057	0,10944	admixed	1
128	Moldaustr. 24, 10319 Berlin near Tierpark	0,13048	0,18259	0,68692	admixed	1
136	Moldaustr. 24, 10319 Berlin near Tierpark	0,2581	0,25296	0,48897	admixed	1
174	Tierpark, Berlin	0,48697	0,40986	0,10316	admixed	1

308	Tierpark, Berlin	0,8438	0,13492	0,02127	admixed	1
310	Tierpark, Berlin	0,45013	0,09578	0,45409	admixed	1
314	Tierpark, Berlin	0,50102	0,45028	0,0487	admixed	1
317	Tierpark, Berlin	0,66602	0,15881	0,17519	admixed	1
320	Tierpark, Berlin	0,22576	0,45684	0,3174	admixed	1
333	12623 Berlin	0,76214	0,22126	0,01663	admixed	1
143	Tiergarten, Berlin	0,82986	0,15939	0,01076	admixed	1
152	Tiergarten, Berlin	0,79801	0,14577	0,05625	admixed	1
166	Tiergarten, Berlin	0,78003	0,16683	0,05316	admixed	1
309	Nordbahnhof park	0,72988	0,1093	0,16079	admixed	1
134	Volkspark Prenzlauerberg, Berlin	0,84225	0,1053	0,05244	admixed	1
142	Volkspark Prenzlauerberg, Berlin	0,62693	0,08785	0,28521	admixed	1
153	Volkspark Prenzlauerberg, Berlin	0,77372	0,0954	0,13087	admixed	1
168	Volkspark Prenzlauerberg, Berlin	0,75805	0,0945	0,14747	admixed	1
170	Volkspark Prenzlauerberg, Berlin	0,35675	0,0564	0,58687	admixed	1
172	Volkspark Prenzlauerberg, Berlin	0,83185	0,10633	0,06181	admixed	1
324	Eisenacher Str.,12629 Berlin near park	0,71554	0,09224	0,19223	admixed	1
300	Kastanienallee 122/126, 12627 Berlin near Teupitzer Park	0,83854	0,12196	0,03952	admixed	1
261	Wolfshofstr. 25, 13591 Berlin	0,20168	0,04025	0,75806	admixed	1
340	Mahlerstraße, 13088 Berlin	0,75765	0,09987	0,14248	admixed	1
241	Glambecker Ring 4, 12679 Berlin	0,66759	0,16157	0,17085	admixed	1
114	Togostr. 45, 13351 Berlin near Volkspark Rehberge	0,61322	0,10559	0,28119	admixed	1
248	13053 Berlin	0,58362	0,0891	0,32725	admixed	1
119	Ghanastr. 27, 13351 Berlin near Volkspark Rehberge	0,7999	0,09627	0,10382	admixed	1
139	Falkenberger Krugwiesen, 13057 Berlin	0,78143	0,11325	0,10533	admixed	1
140	Falkenberger Krugwiesen, 13057 Berlin	0,73941	0,18507	0,0755	admixed	1
150	Buergerpark Pankow-Berlin	0,66583	0,13902	0,19514	admixed	1

188	Schwarzelfenweg 19, 13088 Berlin	0,59525	0,23319	0,17157	admixed	1
116	Alt-Tegel 47c, 13507 Berlin	0,82319	0,14861	0,0282	admixed	1
191	Strasse 26 Nr. 30, 13129 Berlin near green area	0,65149	0,31845	0,03009	admixed	1
196	Schwarzwaldstr., 13129 Berlin	0,67859	0,10107	0,22037	admixed	1
186	Gutenfelsstr. 14, 13129 Berlin	0,81717	0,1626	0,02022	admixed	1
198	Gutenfelsstr. 14, 13129 Berlin	0,76845	0,1193	0,11227	admixed	1
200	Gutenfelsstr. 14, 13129 Berlin	0,8493	0,10802	0,04267	admixed	1
192	Urbacher Str., 13129 Berlin	0,54983	0,25383	0,19634	admixed	1
184	Freischuetzstr., 13129 Berlin	0,81933	0,14711	0,03358	admixed	1
203	Freischuetzstr., 13129 Berlin	0,8327	0,13544	0,03187	admixed	1
197	Krontalerstr., 13125 Berlin	0,84704	0,10899	0,04399	admixed	1
A3_317	Treptower Park	0,77842	0,11654	0,10505	admixed	admixed
A34_078	Treptower Park	0,67359	0,09012	0,23627	admixed	admixed
144	Tierpark, Berlin	0,10456	0,88882	0,00662	2	2
146	Tierpark, Berlin	0,10652	0,8851	0,00836	2	2
154	Tierpark, Berlin	0,10959	0,88179	0,00862	2	2
165	Tierpark, Berlin	0,10523	0,88487	0,00991	2	2
305	Tierpark, Berlin	0,10535	0,88925	0,00539	2	2
306	Tierpark, Berlin	0,10434	0,89067	0,005	2	2
307	Tierpark, Berlin	0,12173	0,85912	0,01916	2	2
312	Tierpark, Berlin	0,10548	0,88768	0,00684	2	2
313	Tierpark, Berlin	0,10518	0,88331	0,0115	2	2
315	Tierpark, Berlin	0,11634	0,86767	0,01601	2	2
318	Tierpark, Berlin	0,11617	0,87305	0,01078	2	2
319	Tierpark, Berlin	0,11028	0,87549	0,01424	2	2
342	Tierpark, Berlin	0,11259	0,87716	0,01028	2	2
344	IZW Garten, Berlin (bordering with Tierpark)	0,10948	0,87852	0,01199	2	2
141	Tierpark, Berlin	0,14033	0,79122	0,06844	admixed	2
149	Tierpark, Berlin	0,10468	0,79688	0,09844	admixed	2
321	Tierpark, Berlin	0,15174	0,82023	0,02802	admixed	2
322	Tierpark, Berlin	0,1618	0,8123	0,02591	admixed	2
157	Treptower Park	0,02233	0,01133	0,96636	3	3

345	Treptower Park	0,01293	0,01151	0,97556	3	3
346	Treptower Park	0,02053	0,00877	0,97068	3	3
348	Treptower Park	0,07228	0,0132	0,9145	3	3
349	Treptower Park	0,0197	0,00997	0,97034	3	3
350	Treptower Park	0,01276	0,01003	0,97721	3	3
A1_317	Treptower Park	0,01622	0,00786	0,97591	3	3
A10_028	Treptower Park	0,02804	0,02522	0,94674	3	3
A11_028	Treptower Park	0,06713	0,02515	0,90771	3	3
A12_028	Treptower Park	0,03142	0,0546	0,914	3	3
A13_028	Treptower Park	0,04447	0,01617	0,93935	3	3
A14_028	Treptower Park	0,01659	0,00572	0,97765	3	3
A15_028	Treptower Park	0,01453	0,01432	0,97112	3	3
A16_028	Treptower Park	0,04624	0,01015	0,94359	3	3
A2_317	Treptower Park	0,03028	0,01088	0,95885	3	3
A20_038	Treptower Park	0,01454	0,01811	0,96735	3	3
A21_038	Treptower Park	0,05401	0,01349	0,9325	3	3
A22_038	Treptower Park	0,04167	0,01371	0,94463	3	3
A25_078	Treptower Park	0,01262	0,02414	0,96325	3	3
A27_078	Treptower Park	0,02487	0,03366	0,94146	3	3
A28_078	Treptower Park	0,01631	0,01948	0,96424	3	3
A30_078	Treptower Park	0,02939	0,01268	0,95794	3	3
A31_078	Treptower Park	0,01652	0,04733	0,93614	3	3
A32_078	Treptower Park	0,01376	0,01006	0,97617	3	3
A37_088	Treptower Park	0,01249	0,01269	0,97483	3	3
A43_088	Treptower Park	0,02567	0,02771	0,94662	3	3
A47_098	Treptower Park	0,01487	0,01031	0,97481	3	3
A5_317	Treptower Park	0,02926	0,0273	0,94345	3	3
A59_108	Treptower Park	0,0119	0,00944	0,97865	3	3
A9_028	Treptower Park	0,00902	0,00582	0,98515	3	3
252	Friedenstr., 16356 Ahrensfelde	0,04766	0,01531	0,93704	3	3
A56_098	Treptower Park	0,12642	0,03009	0,84349	admixed	3
A62_108	Treptower Park	0,13874	0,02767	0,83359	admixed	3
343	Tierpark, Berlin	0,16607	0,05859	0,77535	admixed	admixed

1.7 Acknowledgements

We thank D. Müller and W. Rast for their assistance during field work and for organizing the database, P. Gras for his help with the GIS software and S. Kimmig, I. Möllenkotte and C. Scholz and many others for suggestions to improve the manuscript. We particularly acknowledge all rescue facilities, animal shelters and veterinary surgeries for their support by providing samples and sharing information on the origin of these samples. We also thank the reviewers of the former version for their helpful comments.

1.8 References

Amori, G. 2016. *Erinaceus europaeus*. The IUCN Red List of Threatened Species 2016: e.T29650A2791303. http://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T29650A2791303.en. accessed 18 February 2019.

Belasen AH, Bletz MC, Leite D da Silva, Toledo LF, James TY (2019) Long-Term Habitat Fragmentation Is Associated With Reduced MHC IIB Diversity and Increased Infections in Amphibian Hosts. Front Ecol Evol 6:236 doi: 10.3389/fevo.2018.00236

Andrén, H. (1994). Effects of Habitat Fragmentation on Birds and Mammals in Landscapes with Different Proportions of Suitable Habitat: A Review. Oikos, 71(3), 355. doi:10.2307/3545823

Baker PJ, Harris S (2007) Urban mammals: What does the future hold? An analysis of the factors affecting patterns of use of residential gardens in Great Britain. Mamm Rev 37:297–315

Becher SA, Griffiths R (1997) Isolation and characterization of six polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus*. Mol Ecol 6:89–90

Becher SA, Griffiths R (1998) Genetic differentiation among local populations of the European hedgehog (*Erinaceus europaeus*) in mosaic habitat. Mol Ecol 7:1599–1604

Beninde J, Veith M, Hochkirch A (2015) Biodiversity in cities needs space: A meta-analysis of factors determining intra-urban biodiversity variation. Ecol Lett 18:581–592

Bolfíková BČ, Hulva P (2012) Microevolution of sympatry: landscape genetics of hedgehogs *Erinaceus europaeus* and *E. roumanicus* in Central Europe. Heredity (Edinb) 108:248–255

Bolfíková B, Konečný A, Pfäffle M, Skuballa J, Hulva P (2013) Population biology of establishment in New Zealand hedgehogs inferred from genetic and historical data: conflict or compromise? Mol Ecol 22:3709–3720

Braaker S, Moretti M, Boesch R, Ghazoul J, Obrist MK, Bontadina F (2014) Assessing habitat connectivity for ground-dwelling animals in an urban environment. Ecol Appl 24:1583–1595

Braaker S, Kormann U, Bontadina F, Obrist MKK (2017) Prediction of genetic connectivity in urban ecosystems by combining detailed movement data, genetic data and multi-path modelling. Landsc Urban Plan 160:107–114

Cheng L, Connor TR, Sirén J, Aanensen DM, Corander J (2013) Hierarchical and spatially explicit clustering of DNA sequences with Baps software. Mol Biol Evol 30:1224–1228

Coombs JA, Letcher BH, Nislow KH (2008) Create: A software to create input files from diploid genotypic data for 52 genetic software programs. Mol Ecol Resour 8:578–580

Corander J, Sirén J, Arjas E (2008) Bayesian Spatial Modelling of Genetic Population Structure. Comput Stat 23:111–129.

Doncaster CP, Dickman CR (1987) The ecology of small mammals in urban habitats. I. Population in a patchy environment. J Anim Ecol 56:629–640

Doncaster CP, Rondinini C, Johnson PCD (2001) Field test for environmental correlates of dispersal in hedgehogs *Erinaceus europaeus*. J Anim Ecol 70:33–46

Dowding CV, Harris S, Poulton S, Baker PJ (2010) Nocturnal ranging behaviour of urban hedgehogs, *Erinaceus europaeus*, in relation to risk and reward. Anim Behav 80(1):13–21. doi:10.1016/j.anbehav.2010.04.007

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

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

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinforma Online 1:47-50

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

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

Fischer JD, Schneider SC, Ahlers AA, Miller JR (2015) Categorizing wildlife responses to urbanization and conservation implications of terminology. Conserv Biol 29: 1246-1248

Guo SW, Thompson EA (1992) Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. Biometrics 48:361

Henderson M, Becher SA, Doncaster CP, Maclean N (2000) Five new polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus*. Mol Ecol 9:1949–1951

Herter K (1933) 17.) Gefangenschaftsbeobachtungen an europäischen Igeln II. – Mammalian Biology (formerly Zeitschrift für Säugetierkunde) 8: 195 - 218.

Hof ARA, Bright PPW (2009) The value of green-spaces in built-up areas for western hedgehogs. Lutra 52:69–82

Hof AR, Bright PW (2016) Quantifying the long-term decline of the West European hedgehog in England by subsampling citizen-science datasets. Eur J Wildl Res 62:407–413

Hof AR, Snellenberg J, Bright PW (2012) Food or fear? Predation risk mediates edge refuging in an insectivorous mammal. Anim Behav 83:1099–1106

Hubert P, Julliard R, Biagianti S, Poulle M-LLM (2011) Ecological factors driving the higher hedgehog (*Erinaceus europaeus*) density in an urban area compared to the adjacent rural area. Landsc Urban Plan

103:34–43

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Resour 9:1322–1332

Huijser MP, Bergers PJM (2000) The effect of roads and traffic on hedgehog (*Erinaceus europaeus*) populations. Biol Conserv 95:111–116

Johnson MTJ, Munshi-South J (2017) Evolution of life in urban environments. Science 358(6363). pii: eaam8327

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Krange M (2015) Change in the occurrence of the West European Hedgehog (*Erinaceus europaeus*) in western Sweden during 1950-2010. Karlstad University

Lourenço A, Álvarez D, Wang IJ, Velo-Antón G (2017) Trapped within the city: integrating demography, time since isolation and population-specific traits to assess the genetic effects of urbanization. Mol Ecol 26:1498–1514

Maclagan SJ, Coates T, Ritchie EG (2018) Don't judge habitat on its novelty: Assessing the value of novel habitats for an endangered mammal in a peri-urban landscape. Biol Conserv 223:11-18

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655

Martins RF, Fickel J, Le M, van Nguyen T, Nguyen HM, Timmins R, Gan HM, Rovie-Ryan JJ, Lenz D, Förster DW, Wilting A (2017) Phylogeography of red muntjacs reveals three distinct mitochondrial lineages. BMC Evol Biol 17:34

Miller CR, Joyce P, Waits LP (2002) Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160:357–366

Molony SE, Dowding CV, Baker PJ, Cuthill IC, Harris S (2006) The effect of translocation and temporary captivity on wildlife rehabilitation success: An experimental study using European hedgehogs (*Erinaceus europaeus*). Biol Conserv 130:530–537

Morris PA (1988) A study of home range and movements in the hedgehog (*Erinaceus europaeus*). Journal of Zoology, 214(3), 433–449. doi:10.1111/j.1469-7998.1988.tb03751.x

Morris PA, Reeve NJ (2008) Hedgehog *Erinaceus europaeus*. In: Harris S, Yalden DW (eds.) Mammals of the British Isles: Handbook (4th Ed. pp. 241–248). Southampton, The Mammal Society.

Müller F (2018) Langzeit-Monitoring der Straßenverkehrsopfer beim Igel (*Erinaceus europaeus* L.) zur Indikation von Populationsdichteveränderungen entlang zweier Teststrecken im Landkreis Fulda. Beiträge zur Naturkd Osthessen 54:21–26 (in German)

Munshi-South J, Nagy C (2014) Urban park characteristics, genetic variation, and historical demography of white-footed mouse (Peromyscus leucopus) populations in New York City. PeerJ 2:e310

Opdam, P. (1991). Metapopulation theory and habitat fragmentation: a review of holarctic breeding bird studies. Landscape Ecology, 5(2), 93–106. doi:10.1007/BF00124663

Pitra C, Lieckfeldt D, Frahnert S, Fickel J (2002) Phylogenetic relationships and ancestral areas of the bustards (Gruiformes: Otididae), inferred from mitochondrial DNA and nuclear intron sequences. Molecular Phylogenetics and Evolution 23: 63–74

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

Queller DC, Goodnight KF. 1989. Estimating Relatedness Using Genetic Markers. Evolution 43:258–275. DOI: 10.2307/2409206.

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rautio A, Valtonen, A, Kunnasranta M (2013) The effects of sex and season on home range in European hedgehogs at the northern edge of the species range. Annales Zoologici Fennici, 50(1–2):107–123 doi:10.5735/086.050.0110

Rodríguez-Ramilo S. T., Wang, Jinliang (2012) The effect of close relatives on unsupervised Bayesian clustering algorithms in population genetic structure analysis. Molecular Ecology Resources, 12. Jg., Nr. 5, S. 873-884.

Rondinini C, Doncaster CP (2002) Roads as barriers to movement for hedgehogs. Funct Ecol 16:504–509

Stillfried M, Fickel J, Börner K, Wittstatt U, Heddergott M, Ortmann S, Kramer-Schadt S, Frantz, AC (2017) Do cities represent sources, sinks or isolated islands for urban wild boar population structure? J Appl Ecol 54:272–281

SenUVK: Senatsverwaltung für Umwelt, Verkehr und Klimaschutz Berlin, Referat Freiraumplanung und Stadtgrün (2018), Grünflacheninformationssystem GRIS, https://www.berlin.de/senuvk/umwelt/stadtgruen/gruenanlagen/de/daten_fakten/downloads/ausw_5.pdf; Access January 31st, 2019.

Taylor, A. C., Walker, F. M., Goldingay, R. L., Ball, T., & van der Ree, R. (2011). Degree of Landscape Fragmentation Influences Genetic Isolation among Populations of a Gliding Mammal. PLoS ONE, 6(10), e26651. doi:10.1371/journal.pone.0026651

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: Software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Wahlund S (1928) Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11:65–106 [in German]

Wilcox BA, Murphy DD (1985) Conservation Strategy: The Effects of Fragmentation on Exinction. Am Nat 125:879–887

Wang J (2011) Coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Res 11:141–145.

Zingg R (1994) Aktivität sowie Habitat- und Raumnutzung von Igeln (*Erinaceus europaeus*) in einem ländlichen Siedlungsgebiet. Doctoral thesis, Universität Zürich [in German]

Figure 1.3: Taking a saliva sample from a hedgehog in Treptower Park. Picture by Rohit Chakravarty

2 Application of an SSR-GBS marker system on an investigation of European Hedgehog species and their hybrid zone dynamics

Published in a slightly different version:

Curto M, Winter S, Seiter A, et al. Application of an SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics. Ecol Evol. 2019; 9:2814–2832. https://doi.org/10.1002/ece3.4960

License: Attribution 4.0 International (CC BY 4.0)

Manuel Curto[a], Silvia Winter[a,b], Anna Seiter[a], Lukas Schmid[a], Klaus Scheicher[c] , Leon M. F. Barthel[d], Jürgen Plass[e], Harald Meimberg[a]

[a]Institute for Integrative Nature Conservation Research, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
[b]Division of Plant Protection, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
[c]Institute of Mathematics, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
[d]Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany
[e]Biologiezentrum Linz, Oberösterreich Landesmuseum, Linz, Austria

2.1 Abstract

By applying second-generation sequencing technologies to microsatellite genotyping, sequence information is produced which can result in high-resolution population genetics analysis populations and increased replicability between runs and laboratories. In the present study, we establish an approach to study the genetic structure patterns of two European hedgehog species *Erinaceus europaeus* and *E. roumanicus*. These species are usually associated with human settlements and are good models to study anthropogenic impacts on the genetic diversity of wild populations. The short sequence repeats genotyping by sequence (SSR-GBS) method presented uses amplicon sequences to determine genotypes for which allelic variants can be defined according to both length and single nucleotide polymorphisms (SNPs). To evaluate whether complete sequence information improved genetic structure definition, we compared this information with datasets based solely on length information. We identified a total of 42 markers which were successfully amplified in both species. Overall, genotyping based on complete sequence information resulted in a higher number of alleles, as well as greater genetic diversity and differentiation between species. Additionally, the structure patterns were slightly clearer with a division between both species and some potential hybrids. There was some degree of genetic structure within species, although only in *E. roumanicus* was this related to geographical distance. The statistically significant results obtained by SSR-GBS demonstrate that it is superior to electrophoresis-based methods for SSR genotyping. Moreover, the greater reproducibility and

throughput with lower effort which can be obtained with SSR-GBS and the possibility to include degraded DNA into the analysis, allow for continued relevance of SSR markers during the genomic era.

2.2 Introduction

Second-generation sequencing technologies are revolutionizing not only genome-wide analyses but also genotyping approaches. Several genotyping by sequencing methods have been developed and refined to the point that large parts of the genome can be covered, RAD-sequencing (Restriction Site associated DNA) being the most prominent example (Andrews, Good, Miller, Luikart, & Hohenloh et al., 2016). Additionally, next-generation sequencing (NGS) technologies have a large potential for traditional microsatellite (simple sequence repeat, SSR) analysis (de Barba et al., 2017). Although RAD-sequencing methods are becoming more widely adopted, they still require relatively high coverage per locus and thus high-throughput sequencing (Hodel et al., 2016). With lower coverage, the amount of missing data increases, compromising population genetic analyses of the subsequent datasets (Arnold, Corbett-Detig, Hartl, & Bomblies, 2013; Curto, Schachtler, Puppo, & Meimberg, 2018).

Here, we use the term genotyping by sequencing (GBS) in the context of Elshire et al. (2011) and Vartia et al. (2016), referring to the genotype determination via second-generation sequencing data, Illumina being the most commonly used technology. At its most extreme, GBS is whole-genome analysis applications such as the resequencing of population pools and individuals, as exemplified by the dense SNP genotyping in human population genetics (e.g., 1000 Genomes Project Consortium, 2010; Li & Durbin, 2011) and animal breeding (e.g., Rubin et al., 2010; Daetwyler et al., 2014). As for most systems a reference genome is unavailable, downsizing is required, thus allowing the investigation of only a subset of loci within the genome (Cronn et al., 2012). Examples of these reduced representation approaches are the following: RAD-sequencing (Baird et al., 2008), exon capture (Lemmon, Emme, & Lemmon, 2012), and amplicon sequencing. This last approach is genome downsizing to the largest extent, as only unique regions of the genome, such as single nucleotide polymorphisms (SNPs), are targeted. These methods can be further modified to fit high-throughput approaches, such as with the use of inversion probes or genotyping by the thousand approaches (Campbell, Harmon, & Narum, 2015; Hardenbol et al., 2003).

Amplicon sequencing has a special role in SSR analysis (de Barba et al., 2017; Farrell, Carlsson, & Carlsson, 2016; Vartia et al., 2016; Šarhanová et al., 2018), and microsatellite amplification is the

method of choice for population genetics, due to the ability to recover multiple alleles per locus, resulting in a high statistical power with a low number of sequenced markers (Ellegren, 2004; Schlotterer, 2000). Despite the obvious advantages of whole-genome sequencing approaches, genotyping of specific loci is more cost-effective and more easily implemented, which is also one of the arguments found in recent reviews for the use of microsatellites in place of RAD/GBS (Hodel et al., 2017, 2016). Second-generation sequencing methods facilitate new, more powerful applications using microsatellite loci by increasing the data collected and the possibility to reach high statistical power by increasing the number of markers per sample and the number of alleles per marker (de Barba et al., 2017; Tibihika, Curto et al., 2018; Vartia et al., 2016). Using this method, it is now possible to recover the complete sequence composition of the locus, including the repeat motif and SNPs in the flanking region. This approach makes it possible to overcome homoplasy characteristics of microsatellites (Vartia et al., 2016; Šarhanová et al., 2018). In these cases, shared alleles resulting from homoplasy would have the same number of repetitions but different flanking regions. Additionally, the application of GBS to SSR markers (SSR-GBS) leads to an improvement in the reproducibility of data produced by different laboratories. Although problems caused by stutter bands remain, limitations associated with machine-specific biases, the need to use the same size standards or the 'plus A peak' artefact do not apply to SSR-GBS. For these reasons, SSR markers are one of the most promising and obvious choices for GBS applications, and SSR-GBS has the potential to overcome some of the shortcomings associated with traditional microsatellite analysis when compared to RADs (Hodel et al., 2017, 2016).

The primary advantage of RAD-seq is the high number of SNPs that can be detected across the genome with relatively low cost and without previous genomic information (Andolfatto et al., 2011; Smith et al., 2010; Sonah et al., 2013). The high number of loci recovered with RAD-seq allows for the recovery of population genetic differentiation patterns (Schopen, Bovenhuis, Visker, & Van Arendonk, 2008). However, there are some limitations associated with RAD-seq, such as the difficulty in detecting paralogs without a reference genome, the high amount of missing data, and biases caused by the use of restriction enzymes that influence heterozygosity estimates, especially when stringent data filtering is implemented (Hodel et al., 2017). Further, SSR markers' costs and data collection efforts do not increase linearly as a function of sample size. This compares favourably to RAD-seq when genotyping high numbers of individuals (in the order of thousands), or for short-term projects (Hodel et al., 2016). With the lower costs of the SSR-GBS approach, this advantage is expected to be even greater. In this respect, SSR-GBS has similarities with the genotyping by the thousands approach (Campbell et al., 2015).

In this paper, we present the development of SSR markers and their application in multiplexed amplifications to measure genetic variation in two species of hedgehogs: the European hedgehog (*Erinaceus europaeus*) and the northern white-breasted hedgehog (*Erinaceus roumanicus*). Both species occur in Austria where their ranges form a contact zone. These ranges are classic examples of postglacial recolonization patterns and the formation of a secondary contact zone in response to this process (Hewitt, 1999; Santucci, Emerson, & Hewitt, 1998). It has been hypothesized that during the glacial periods, populations which found refuge in the Iberian and Italian peninsulas diverged from a common ancestor to *E. europaeus*, while those in the Balkans to *E. roumanicus* (Seddon, Santucci, Reeve, & Hewitt, 2001). Both species are closely related, but hybridization seems to only occur occasionally (Bogdanov, Bannikova, Pirusskii, & Formozov, 2009) and molecular markers support a clear genetic division between the two species when they occur in sympatry (Bolfíková & Hulva, 2012). Thus, according to current knowledge, these species do not form a hybrid zone. However, all previous investigations of hybridization between these species performed thus far were based on a low number of markers. Both species seem to be generally present among human settlements (primarily in gardens/yards), but in the contact zone distribution of both species might be influenced by competition. Regardless, hedgehogs are species that are potentially impacted by fragmentation of their habitat by human infrastructures, roadways potentially being the most significant barriers for gene flow and migration (Huijser & Bergers, 2000; Orlowski & Nowak, 2004). These hedgehog species have a moderate genetic structure, and on a larger scale, they show isolation by distance pattern that is likely a consequence of recolonization after the last glaciation period (Bolfíková et al., 2017; Seddon et al., 2001). However, it has been verified that on small spatial scales the isolation by distance pattern can be disturbed due to habitat fragmentation and anthropogenic barriers to gene flow (Becher and Griffiths 1998), hence the importance of studying the genetic variation of these species in restricted geographical scales (Braaker, Kormann, Bontadina, & Obrist, 2017).

Second-generation sequencing technologies provide new opportunities, in particular in studies where several species are examined. By increasing the information provided by genetic markers, one can detect genetic structure at smaller geographical scales and may be able to detect residual signs of hybridization that would otherwise be undetected (Corander & Marttinen, 2006; Ryman et al., 2006). Traditionally, microsatellite markers used in cross-species amplification could potentially lead to bias favouring the species from which the markers originated (Turini et al., 2014). Additionally, biases in variability are also possible, which stem from modification, interruption or shortening of the repeat (Callen et al., 1993; Varshney, Graner, & Sorrells, 2005).

Therefore, in addition to mismatches at the primer site leading to an increase in null alleles, markers might show less variability when used in cross-species amplification.

Taking advantage of the Illumina technology, we developed markers from both species and tested their ability to amplify cross-species markers. We determined the effectiveness of marker multiplexing to facilitate data collection and tested genotyping with the Illumina, using both length and sequence information in an SSR-GBS approach, with tissue as well as noninvasive sampling, and outlined the results of genetic structure. The dataset we present here will form the basis of comprehensive studies of hedgehog genetic diversity, as well as investigations of introgression and gene flow between populations of the same and different species. Phylogeographic implications are outlined.

2.3 Material and methods

2.3.1 Sampling and DNA isolation

A total of 82 individuals were used in the current study, 41 were identified as *E. europaeus* and 41 as *E. roumanicus* (Supplementary Table 2.1). While most individuals were sampled in Austria, some were collected in other locations: one in Berlin, two in southeast Germany (Bavaria) near the border with Austria, two in eastern Slovakia, five in southwestern Czech Republic, one in northwestern Croatia, one in Hungary, and one in Macedonia. Sampling in Austria was concentrated in the areas surrounding Linz (35). Within this area, we subdivided the samples into four sub-regions: Southeast Linz (3), East Linz (5), Linz (13), and West Linz (14). Four samples were collected in the areas surrounding Vienna in the province of Lower Austria, three of them in the region east of the city and one west of the city. Six samples were from southeast Austria in the province of Burgenland, five of them collected east of the lake Neusiedlersee. Twenty-four samples were collected by three animal shelters: seven in Bludenz (Vorarlberg) and in Innsbruck (Tirol) in western Austria and 10 in Klagenfurt (Carinthia) in southern Austria. According to information from the shelters, these individuals were found within 100 km radius of the shelter and within the same province. Shelter samples were collected using mouth swabs from live animals, with the remaining ones collected as tissue samples from road fatalities. Individual samples were collected by several institutions (Supporting Information Table S1): the Biologiezentrum Linz, the Natural History Museum in Vienna, Leibniz Institute for Zoo and Wildlife Research, and the animal shelters.

For DNA isolation of buccal swabs, the swabs were placed in 500 µl lysis buffer (2% SDS, 2% PVP-40, 250 mM NaCl, 200 mM Tris-HCl, 5 mM EDTA, pH 8) and 16.67 µl of proteinase K

(10 mg/ml) and incubated for 2.5 hr at 56°C. They were then removed with clean tweezers and placed in a NucleoSpin filter columns and centrifuged for 1 min at 562 g. For DNA purification, 400 µl of the supernatant were mixed with 15 µl of MagSi-DNA beads (size 300 nm, MagSi-DNA beads from MagnaMedics) and 600 µl binding buffer (2 M GuHCl in 95% ethanol) and incubated at room temperature for 5 min. The supernatant was separated from the beads by placing samples on the magnetic separator SL-MagSep96 (Steinbrenner, Germany) for one minute. The beads were washed twice with 600 µl of 80% ethanol. To remove excess ethanol, the beads were air-dried at room temperature for 10 min. Two elutions were made with 20 and 25 µl preheated (65°C) elution buffer (10 nM Tris with a pH of 8), and the beads were mixed with elution buffer and incubated for 5 min at room temperature. Tissue samples were isolated by the same procedure, with the exception that the product of lysis required no filtration, and the DNA was eluted in 30 and 50 µl of elution buffer.

2.3.2 Marker development

Marker development was conducted using two low-coverage MiSeq runs, where one individual each of *E. europaeus* and *E. roumanicus* were sequenced using shot-gun genomic libraries without enrichment. The *E. roumanicus* sample was roadkill from Romania. The *E. europaeus* sample stems from a sample collected in the area of Berlin. Both runs produced 300 bp paired-end reads using libraries prepared with an insert length of between 400 and 500 bp to allow for sequence overlap. Raw reads of both runs are available in GenBank's SRA repository with the accession number PRJNA495814. Low-quality regions and adapter sequences were trimmed using Cutadapt v. 0.11.1 (Martin, 2011), and the resulting reads were merged using PEAR vers. 0.9.4 (Zhang, Kobert, Flouri, & Stamatakis, 2013). These merged reads were used as input for the SSR_pipeline's script SSR_search.py in order to determine which sequences contained SSR motifs (Miller, Knaus, Mullins, & Haig, 2013). The following steps of quality control were included: The sequence contained a minimum of 40 bp flanking both sides of the motif; a minimum of six repeats for tetra- and pentanucleotide; a minimum of eight repeats for trinucleotides; and 10 repeats for dinucleotides. The number of sequences generated in the size range (350–550 bp) was sufficient for extracting a large number of microsatellite motif-containing sequences. Sequences containing interruptions of the motif and mononuclear stretches larger than six bp were manually excluded; however, for some motif types this step resulted in a too low number of usable reads was not feasible, and in these cases some mononucleotide repeats were accepted.

Primers were constructed using Primer3 (Untergasser et al., 2012) as implemented in Geneious v. 8.1.8 (Kearse et al., 2012) as a batch job under manual control. We only retained primers which produced amplicons containing the complete microsatellite repetition motif in the first or last 300 bases. This allowed the merging of paired reads in 300 bp MiSeq runs. Primers were designed to be between 19 and 22 bp long, with an optimal melting temperature of 55 ºC. These were elongated with a recognition sequence that corresponded to the Illumina adapter, the forward primer being elongated with part of the P5 motif (TCTTTCCCTACACGACGCTCTTCCGATCT) and the reverse with part of the P7 motif (CTGGAGTTCAGACGTGTGCTCTTCCGATCT). These recognition sequences are necessary for a second PCR where eight-bp index information and the rest of the Illumina adapters are added (P5: AATGATACGGCGACCACCGAGATCTACAC [Index] ACACTCTTTCCCTACACGACG; and P7: CAAGCAGAAGACGGCATACGAGAT [Index] GTGACTGGAGTTCAGACGTGT). Adapters were designed according to the Truseq chemistry because our initial experiments predated the release of the Nextera Chemistry that Illumina recommends for amplicon sequencing. For new experiments using this approach, the Nextera adaptors should be used.

2.3.3 SSR-GBS amplicon library preparation

Primers were first tested individually in 10 µl PCRs containing 5 µl of QIAGEN Multiplex PCR Master Mix (Qiagen, CA, USA), 4 µl of each primer (1 µM), and 1 µl of template/genomic DNA. PCR was conducted using the following temperature profile: 95 ºC for 15 min; 30 cycles of 95 ºC for 30 s, 55 ºC for 1 min, and 72 ºC for 1 min; and a final extension at 72 ºC for 10 min. PCR results were visualized using agarose gel electrophoresis, and primers which amplified a fragment of the correct size were combined in several primer mixes.

For genotyping, three runs were performed using relevant samples. The first included two samples which were amplified using different multiplex approaches: singleplex, and multiplexes of 4 and multiplex of 10 primer pairs, with the 35 *E. roumanicus* primer pairs. The 10 primer pair multiplex PCR was able to recover all loci; therefore, this approach was applied for the following runs. These comprised the same mixes of the *E. roumanicus* primers as above and a single mix of all *E. europaeus* primers. Primer mix solutions for multiplex PCR were composed of a combination of 10 to 30 primer pairs, each primer having a final concentration of 1 µM (Supplementary Table 2.2). Multiplex amplification was performed using a protocol adapted from Curto et al. (2013). PCRs contained 0.5 µl of primer mix, 1 µl of DNA, 5 µl of QIAGEN Multiplex PCR Master Mix and water to complete the final reaction volume of 10 µl. All

amplifications were performed using the same temperature profile as the single PCRs. PCR products from different primer mixes were mixed in equal volumes for each sample. This was primarily done to save time and cost, and a comparison with earlier experiments, where only a few primers were kept in multiplex (around 10), did not show an obvious change in the rate of success (e.g., increased dropout of loci and alleles).

Before proceeding to the second PCR, unused primers and primer dimer constructs were removed from the first PCR. PCR clean-up was performed using magnetic bead technology following the protocol from Agencourt AMPure XP PCR Purification with some slight modifications. Four microlitres of PCR product was mixed with 2.86 µl of AMPure XP beads (Beckman Coulter Inc., Bree, CA, USA) and incubated for 5 min at room temperature. Bound DNA beads were captured by an inverted magnetic bead extraction device, VP 407-AM-N (V&P Scientific, INC.) and washed twice in an 80% 200 µl ethanol solution for 45 s. Later, the beads were dried at room temperature for 5 min and eluted in 17 µl of elution buffer (65 ºC 10 mM Tris-HCl, pH 8.3).

For the second PCR, a unique combination of forward and reverse indexes were chosen, allowing unambiguous identification of each sample after the MiSeq run. The PCR was conducted in a total volume of 10 µl containing 2 µl of each primer (1 µM), 5 µl of QIAGEN Multiplex PCR Master Mix, and 1 µl of purified PCR product. The reaction was carried out, after an initial denaturation and activation at 95ºC for 15 min, using 10 cycles of 95ºC for 30 s, 58ºC for 60 s, and 72ºC for 60 s. The reaction was incubated at 72°C for 5 min as a final extension. The resulting product consisted of the following from 5' to 3': (a) P5 motif for flow cell hybridization, (b) index 1 consisting of 8 bp, (c) P5 sequencing primer, (d) specific forward primer, (e) target DNA for sequencing; specific reverse primer; (f) P7 sequencing primer, (g) index 2 consisting of 8 bp, and (h) P7 motif for flow cell hybridization. In total, 10 different Index 1 and 10 different Index 2 sequences were used, allowing 100 different libraries to be sequenced simultaneously. PCRs were visualized on a 1.8% agarose gel and then pooled in equal volumes. Measurement of the DNA concentration was not performed as the fluctuation in DNA content within one Multiplex reaction was higher than between two reactions; it was therefore assumed that normalization would not change the overall performance.

The resulting pool was used as input for an Illumina MiSeq run to produce sequences used for a genotyping by sequencing procedure. The pool, ca. 100 µl, was purified with magnetic bead technology, as described above, to remove possible dimers prior to Illumina sequencing. The amplicon libraries were sequenced in three runs with calculated yields between 7.5 and 30 K

sequences per DNA sample over all markers assuming an average of 15 M reads from a MiSeq run. Thus, it was expected that between 250 and 1,000 sequences per locus per sample would be obtained.

2.3.4 **Sequence data extraction**

The Illumina run was analysed to determine sample genotypes in different steps (Figure 2.1). Extractions according to index combinations were automatically performed by the MiSeq machine, resulting in two fastq files containing all sequences per index, one for Read 1 and the other for Read 2. A combination of custom made scripts and third-party programs was used for further processing of the samples, including quality control and trimming, merging of the paired reads, identification of primer sequences on both sides of sequences, and splitting the files according to primer sequences. Custom scripts were also used (Tibihika, Curto et al., 2018) and are available at github.com/mcurto/SSR-GBS-pipeline. First, paired reads were merged and quality controlled using the program PEAR. Reads were only merged if they overlapped for at least 10 bp with a p-value below 0.01 for the highest observed expected alignment scores (OESs according to Zhang et al., 2013). Unmerged reads were not analysed further. Merging was only possible because primers were designed to allow the complete microsatellite repetition motif to be sequenced by one of the paired reads. By doing so, it was also possible to assess the amplicon length. Previous to merging, low-quality regions (Phred <20) were trimmed. In a second step, script 1 was used to identify the primer sequences on both sides of the merged reads and then sort them according to the locus. According to our library preparation construct, the merged reads should start with the forward primer and end with the reverse primer sequence. All sequences not containing both primer motifs in the correct position were excluded. This step saved all sequences in one file by locus and sample. These files were used as input for subsequent genotyping analysis.

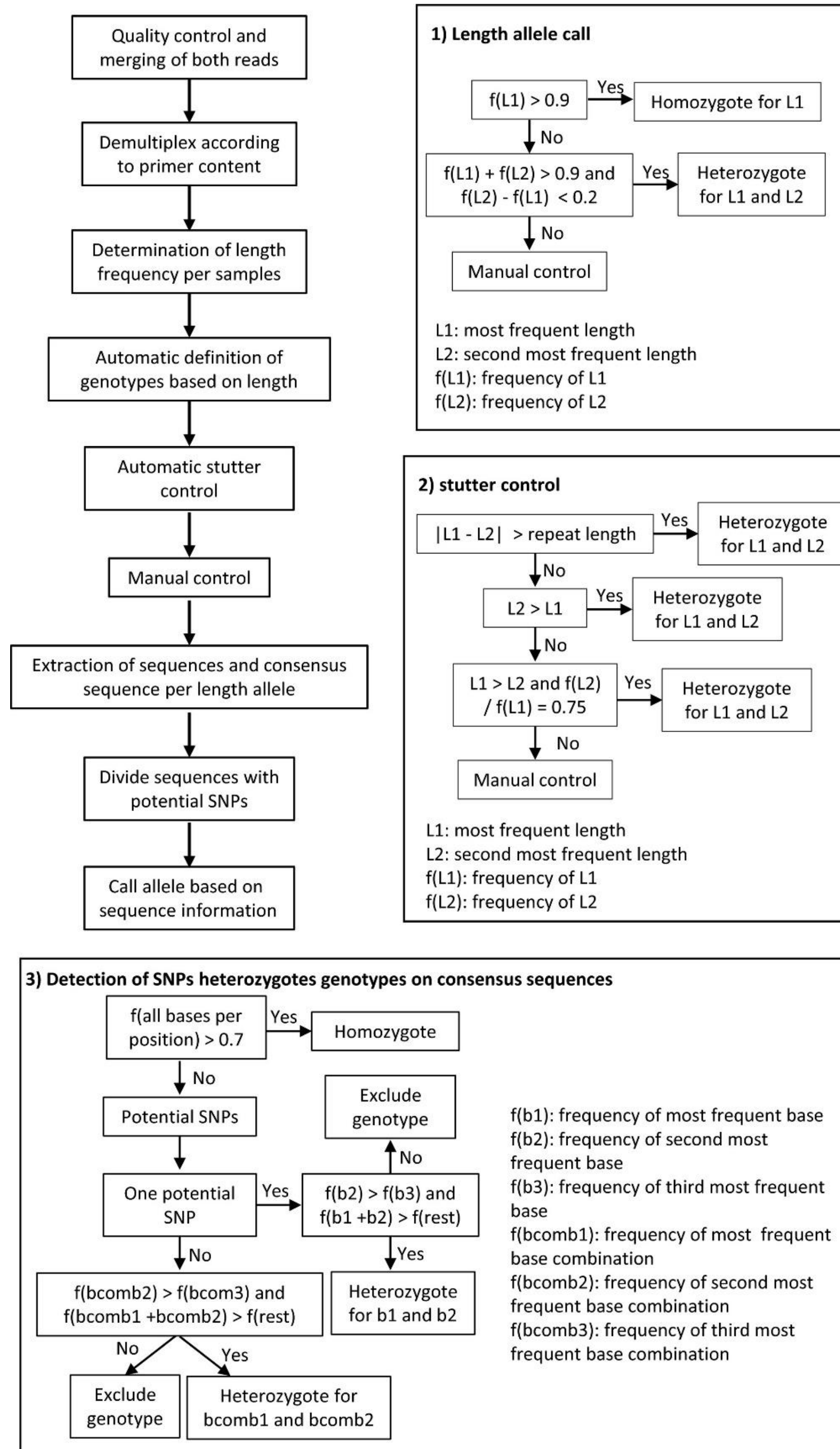


Figure 2.1: Summary of sequence analysis and genotyping approach. The top left panel shows an overview of the method. The right and bottom panels show decision trees concerning: allele call based on length (1), stutter control step (2), detection of SNP genotypes (3). L1 and L2 correspond to the two most frequent lengths found per sample and marker, while f(L1) and (L2) to their frequency. f(b1), f(b2), and f(b3) correspond to the frequencies of the most, second most and third most frequent nucleotides per position, respectively. f(bcomb1), f(bcomb2), and f(bcomb3) correspond, respectively, to the frequencies of the most, second most and third most frequent nucleotides combinations of two or more potential SNPs

2.3.5 Allele definition

Alleles were defined based on the length of sequences and then on the occurrence of SNPs within each length class (Figure 2.1). With script 2 (Supplementary information Chapter 2: Application of an SSR-GBS marker system on an investigation of European Hedgehog species and their hybrid zone dynamics), the sequence lengths occurring in one file and their corresponding counts were calculated and saved. Subsequently, all sequences with a length below a threshold (300 bp) were excluded from genotyping. Amplicons were constructed to be larger than 400 bp, so length of markers below this read length was likely artefacts and was excluded. Potential alleles were classified based on their length frequency using script 3 (Figure 2.1). Loci comprising one length with a frequency equal to or >90 % of all reads were called homozygous for an allele characterized by the respective length. Genotypes were called heterozygous if the frequency of two lengths was >90 % of reads and if the frequency of both lengths differed by no more than 20 % (Figure 2.1). In a second step, the script 3 verified that the selected alleles were not the result of stutter. This was performed using the following three criteria (Figure 2.1): (a) the difference in length of the potential alleles is greater than one time the repeat motif length; (b) If condition one is not met, that is, if the two alleles differ by only one repeat, the allele of lower frequency must be longer than the one of higher frequency; (c) if condition two is not met, that is, if the two alleles differ in one repeat and the frequency of the shorter allele is lower than the frequency of the longer allele, then the shorter allele must have a frequency of 75% of the longer one. In Figure 2.1, we show one example of each case. The criteria were chosen in-line with procedures used for allele calls based on chromatographic data. Programs (e.g., Genemapper, ABI as discussed in Johansson, Karlsson, & Gyllensten, 2003) frequently use the highest signal for allele call. In case of stutter bands in heterozygotes, the signal of the shorter allele and of a stutter band of the longer allele will be overlaid. This can lead to the shorter allele in a heterozygote having a stronger signal (or higher frequency in our case) than the longer allele. Our criteria take this into consideration and call a heterozygote if the stutter band pattern of a homozygote is interrupted (I), if one allele is potentially overlaid by stutter bands (II and III). After automated allele call, all data were plotted into histograms resulting in a graphic representation similar to traditional SSR chromatograms. This allowed for manual control of the allele call like standard for analysis using Genmapper or similar software (Meimberg et al., 2006). With this, our approach could be performed analogously to traditional fragment analysis. Generally, we were able to control for unspecific products. The typical stutter pattern of the homozygote genotypes and resulting from this the length frequency profile should look similar to a heterozygote genotype with overlaid stuttering. Only dinucleotide repeats required that a

larger number of alleles be manually corrected. For penta-, tetra-, and trinucleotide repeats, the number of errors was very low and few corrections were necessary. All steps up until the geographical representation of frequencies and the table of genotypes according to length can be run automatically using the wrapping script *microsatPip*.

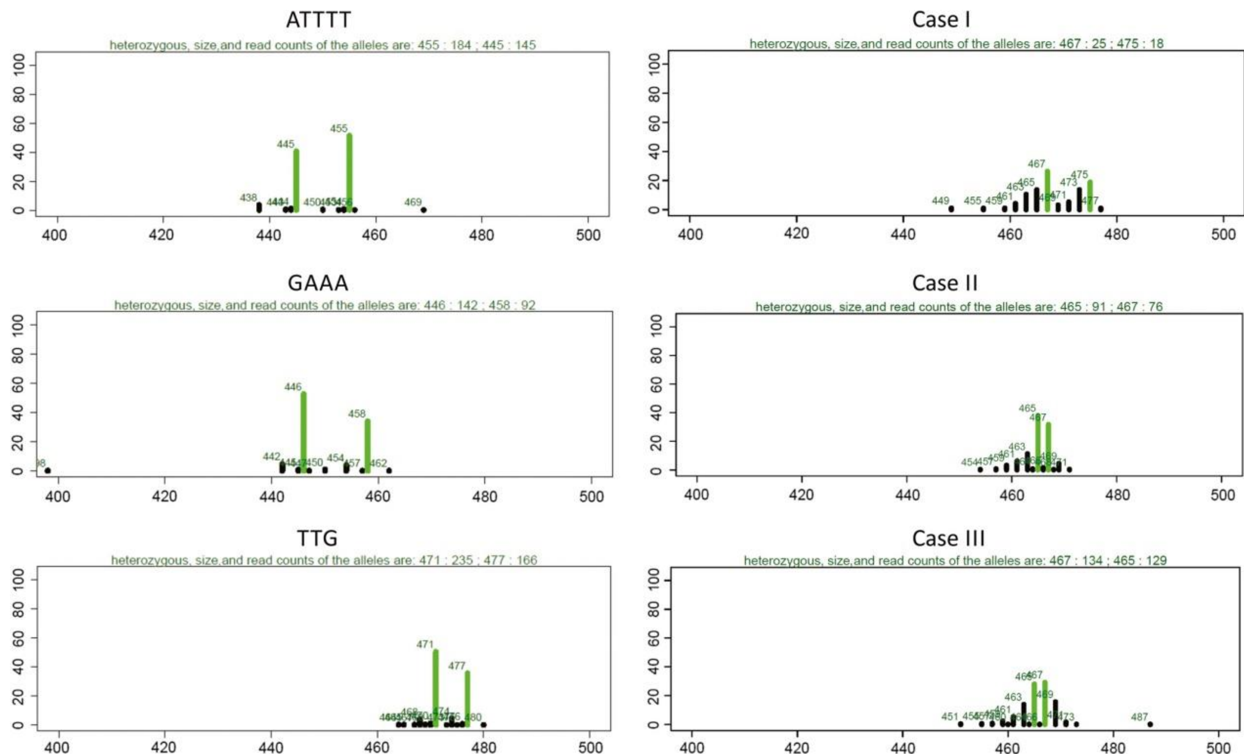


Figure 2.2: Number of reads per amplicon length. The left panel shows unambiguous heterozygote genotypes for tri-, tetra-, and pentanucleotide motifs. The right panel shows examples matching the three cases of the automatic stutter control: Case I, two alleles with a length difference above the repetition motif length; case II, two alleles with length difference equal to the motif length, whose the frequency of the shorter is higher than the longer one; case III, two alleles with length difference equal to the motif length, whose the frequency of the shortest allele is more than 75% of the longer one. Green bars correspond to amplicon lengths chosen as alleles by the genotyping method. Numbers above each bar indicate the allele length. The line above each graph indicates the chosen genotype and the corresponding number of reads supporting it

After manual control, sequences corresponding to the alleles based on length, were separated using the script 4 and condensed into one consensus sequence using the script 5. Frequencies of the most frequent nucleotide per position above 70% were considered homozygous and below 70% as potentially heterozygous. These heterozygous positions were indicated as ambiguous bases on the consensus sequence. For these cases, the consensus sequence was divided into two sequences based on the two most frequent nucleotides for that position using the script 6 (Figure 2.1; Supplementary Table 2.3). In the event that more than one SNP occurred in a sequence, these positions were considered as linked and the two most frequent nucleotide combinations were selected. If more than two equal frequency nucleotide combinations were found, the SNPs were either called by hand or left as ambiguous positions. In case this sample was already heterozygous for allele length, only the most frequent SNP combination was chosen. This

approach was adopted under the assumption that sequencing errors and PCR errors such as chimeric sequences are less frequent than the sequences stemming from real alleles. For allele calling using the complete sequence information, each unique sequence (allele) was given a number and, according to which sequence was present for each sample, a codominant matrix was created. This was done using script 7. For comparison, the same was done with sequence length information, which was obtained after correcting the matrix produced by script 3 (Supplementary Table 2.3).

2.3.6 **Population genetics analysis**

Population genetic analyses were performed using the codominant matrix as input with different standard programs. The dataset was analysed for marker variability and polymorphism information content, as well as for genetic structure patterns among samples.

Variability measures per markers and population, such as number of alleles (Na) and observed (HO) versus expected (HE) heterozygosity, were calculated in GenAlEx v. 6.5 (Peakall & Smouse, 2006). Polymorphism information content (PIC) was obtained with the program Cervus v. 3.0.7 (Kalinowski, Taper, & Marshall, 2007). For comparison between genotyping approaches (length vs. complete sequence information) and primer sets (*E. europaeus*- or *E. roumanicus*-specific primers), we also calculated genetic distances among individuals. This consisted of the average number of differing alleles per locus between each pair of samples. This was done using pairwise distance matrices containing the total number of different alleles per sample calculated with GenalEx. To facilitate graphical visualization, genetic distances were converted into average number of different alleles per locus. Differences between genotyping methods and marker sets for all above-mentioned statistics were tested using the t-tests as implemented in R v. 3.5.1 (R Core Team, 2018).

To evaluate genetic structure between species and populations without assumptions of Hardy–Weinberg Equilibrium (HWE), absolute genetic distances between individuals were calculated and the resulting matrix was used in a principal coordinates analyses (PCoA) as it is implemented in GenAlEx. This analysis was performed first using the complete dataset and then using only individuals from each species. All genetic structure analyses were done using both length and sequence information to test if the additional SNP information contributed to a more detailed genetic diversity pattern.

Sample clustering was evaluated using STRUCTURE v. 2.3.4 (Hubisz, Falush, Stephens, & Pritchard, 2009). This was done for datasets consisting of all samples, only *E. europaeus* and only

E. roumanicus. To evaluate if genetic structure was affected by the use of species-specific markers, STRUCTURE analyses were performed using either markers specifically designed for *E. europaeus* or *E. roumanicus*. Both length- and sequence-based genotyping was used for these analyses. STRUCTURE was run using 15 independent replicates for 500,000 generations after a burn-in period of 100,000. The admixture model and the allele frequencies among samples were considered to be correlated. K-values between 1 and 10 were tested, and the K-value was evaluated through the Delta-K method implemented in the online program Structure Harvester, available at http://taylor0.biology.ucla.edu/structureHarvester/ (Earl, 2012). Replicates per K-value were summarized using the online pipeline Clumpak (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015) available at http://clumpak.tau.ac.il/. To evaluate possible isolation by distance, a Mantel test was performed in GenAlEx comparing geographical and genetic distance matrices among individuals using the data produced from sequence information.

2.4 Results

2.4.1 Maker development

For marker development, the MiSeq runs resulted in 2,201,005 and 1,348,477 paired reads for *E. roumanicus* and *E. europaeus*, respectively. After quality control and merging, a total of 1,464,370 and 716,091 reads were available for microsatellite motif screening. In total, 70,704 and 8,677 microsatellite-containing sequences passed our criteria for *E. roumanicus* and *E. europaeus*, respectively. From these, there were 32,466 dinucleotide, 9,966 trinucleotide, 26,249 tetranucleotide, and 2,023 pentanucleotide repeats for *E. roumanicus*. For *E. europaeus*, there were 4,175 dinucleotide,730 trinucleotide, 3,539 tetranucleotide, and 233 pentanucleotide repeats. In total, 37 primers were designed for *E. roumanicus* and 34 for *E. europaeus*. Of these, 12 failed in the initial amplification step. The remaining primers are listed in Supplementary Table 2.2.

2.4.2 Sequence analysis and genotyping

The three runs resulted in a total of 196,165, 842,591 and 1,790,852 paired reads, respectively. After quality control, paired read merging and primer demultiplex, 4,232,682 reads remained for all three runs. For each marker, the number of sequences varied between 268 and 446,616 per marker and between 12,664 and 136,247 per sample. The marker with the lowest number of sequences was W25_TTA and the one with the highest was W31_GA. Only 10 markers were not retained after the multiplex step: E25_TAC, E6_AAT, E32_ATCT, W20_TAGA, W24_ATA, W25_TTA, W26_TAT, W27_ATA, W3_AAAGA, and W5_AAAAT. These markers were not

considered further despite based on singleplex reaction tests, they would have been able to be measured in less complex multiplex reactions.

Even though most markers were able to be amplified in both species, variability in the species from which they were not derived (non-target species) was lower for many markers (Supplementary Table 2.4). In five markers, the motif was missing in the non-target species, and in three additional markers, the motif was interrupted and was less variable. In a few cases, alleles were fixed. In only a single case was a marker derived from *E. roumanicus* fixed in *E. roumanicus* but variable in *E. europaeus*. We excluded markers that were unable to produce genotypes for most samples (missing data >50%). This resulted in a total of 42 markers for further analysis. When only one species was analysed after excluding markers based on missing data, only 42 markers remained for *E. europaeus* and 41 for *E. roumanicus*. Samples stemming from mouth swabs and tissue material contained on average 31% and 16% missing data, respectively. This corresponded to significantly higher missing data for mouth swabs samples when compared to tissue samples.

2.4.3 **Marker variability**

Markers had between 1 and 23 alleles when only length polymorphisms were considered (Supplementary Table 2.4; Table 2.1). When sequence information was included these numbers varied between 1 and 50 alleles. This corresponded to an increase in the number of singletons (72 for length and 196 for sequence information) and alleles shared among 2–10 individuals (Length = 181, Sequence = 327; Figure 2.3). There was no change in the number of alleles shared among 11 and 20 samples (86), while the allele call based on sequence information contributed to a decrease in the number of alleles shared among 21 or more individuals (Figure 2.3). One marker was monomorphic for the complete dataset including SNPs (E24_GCA) and two more were monomorphic in *E. roumanicus* (W15_ATAA) or *E. europaeus* (W13_TTTA). Considering length information and excluding the monomorphic markers, HO varied between 0.09 and 1.00, HE between 0.25 and 0.94, and PIC between 0.23 and 0.93. Including sequence information, HO varied between 0.12 and 1.00, HE between 0.49 and 0.97, and PIC between 0.46 and 0.96. The number of alleles within *E. europaeus*, excluding monomorphic markers, varied between 2 and 17 for length information and between 3 and 28 for sequence information. For the allele length dataset, HO varied between 0 and 1.00, HE between 0.05 and 0.89, and PIC between 0.05 and 0.87. When considering sequence information, the same values varied between 0.03 and 1, 0.07 and 0.94, and 0.11 and 0.94, respectively. For *E. roumanicus*, the number of alleles, excluding monomorphic markers, varied between 2 and 16 for length information and

between 3 and 34 for sequence information. For the allele length dataset, HO varied between 0 and 1.00, HE between 0.07 and 0.92, and PIC between 0.07 and 0.91. When considering sequence information, the same values varied between 0 and 1, 0.11 and 0.96, and 0.07 and 0.93, respectively.

Table 2.1: Average, across used loci, of amplification success shown as percentage of missing data and average variability measures: N_a—number of alleles, H_O—observed heterozygosity, H_E—expected heterozygosity, and PIC—polymorphism information content. Values in brackets correspond to minimum and maximum values. Values calculated based on sequence information are represented by the superscript 'S' while the ones based on length information by 'L'. Statistics were calculated based on different markers and samples sets

Statistics	Marker set	All samples	*E. europaeus*	*E. roumanicus*
% missing	All	15.48 (0–47.56)	14.75 (0–85.37)	16.2 (0–85.37)
	E. europaeus	15.39 (0–47.56)	18.01 (0–85.37)	12.76 (0–60.98)
	E. roumanicus	15.62 (0–45.12)	9.45 (0–51.22)	21.8 (0–85.37)
N_a^L	All	9.98 (2–23)	7.12 (2–17)	7.1 (2–16)
	E. europaeus	8.96 (3–19)	6.38 (2–13)	6.65 (3–13)
	E. roumanicus	11.63 (2–23)	8.31 (2–17)	7.81 (2–16)
N_a^S	All	16.83 (4–50)	10.45 (3–28)	10.38 (3–34)
	E. europaeus	15.58 (5–49)	9.54 (3–28)	10.15 (3–25)
	E. roumanicus	18.88 (4–50)	11.94 (3–23)	10.75 (4–34)
H_o^L	All	0.45 (0.09–1)	0.44 (0–1)	0.46 (0–1)
	E. europaeus	0.39 (0.09–0.97)	0.35 (0–0.97)	0.43 (0–0.98)
	E. roumanicus	0.55 (0.12–1)	0.59 (0.1–1)	0.51 (0.1–1)
H_o^S	All	0.52 (0.12–1)	0.51 (0.03–1)	0.51 (0–1)
	E. europaeus	0.47 (0.12–0.99)	0.44 (0.03–0.97)	0.49 (0–1)
	E. roumanicus	0.59 (0.12–1)	0.61 (0.1–1)	0.55 (0.12–1)
H_E^L	All	0.74 (0.25–0.94)	0.6 (0.05–0.89)	0.64 (0.09–0.92)
	E. europaeus	0.72 (0.47–0.92)	0.51 (0.05–0.89)	0.64 (0.09–0.9)
	E. roumanicus	0.76 (0.25–0.94)	0.74 (0.31–0.89)	0.65 (0.13–0.92)
H_E^S	All	0.81 (0.49–0.97)	0.68 (0.07–0.94)	0.71 (0.11–0.96)
	E. europaeus	0.8 (0.57–0.95)	0.62 (0.07–0.94)	0.71 (0.11–0.94)
	E. roumanicus	0.83 (0.49–0.97)	0.78 (0.41–0.94)	0.71 (0.16–0.96)
PIC^L	All	0.7 (0.23–0.93)	0.56 (0.05–0.87)	0.6 (0.09–0.91)
	E. europaeus	0.68 (0.37–0.91)	0.48 (0.05–0.87)	0.59 (0.09–0.86)
	E. roumanicus	0.74 (0.23–0.93)	0.7 (0.29–0.87)	0.61 (0.12–0.91)
PIC^S	All	0.78 (0.46–0.96)	0.67 (0.11–0.94)	0.64 (0.07–0.93)
	E. europaeus	0.77 (0.48–0.94)	0.67 (0.11–0.9)	0.58 (0.07–0.93)
	E. roumanicus	0.81 (0.46–0.96)	0.67 (0.16–0.94)	0.74 (0.38–0.92)

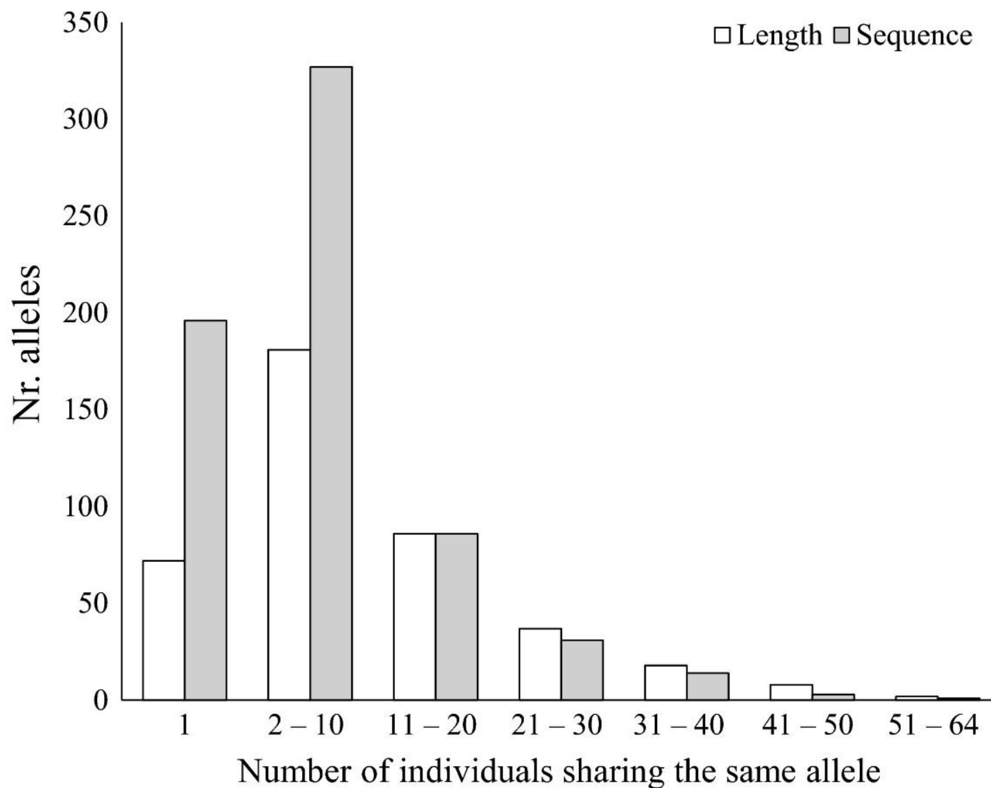


Figure 2.3: Number of alleles shared among individuals shown as the number of alleles (*y*-axis) in dependence to the number individuals that share one allele (*x*-axis). White and grey bars represent alleles called using sequence length information, respectively. The comparison includes the final 41 markers for all 82 individuals

2.4.4 Comparison between genotyping approaches and species-specific primers

Variability per marker was higher when sequence information was considered for allele calling (Figure 2.4). This difference was significant ($p < 0.05$) for all comparisons using Na and for HE and PIC when all samples were considered. Distance among individuals was calculated based on the average number of different alleles per marker between and within each species. Distance between species varied between 0.95 and 3.32 for length information and between 1.05 and 3.32 for sequence information. Among *E. europaeus* samples, distance ranged from 0.78 to 3.17 for length information and from 0.80 and 3.27 for sequence information. Among *E. roumanicus*, it varied between 0.32 to 3.10 for length information and between 0.41 and 3.22 for sequence information. As shown in Figure 2.5, distance was higher between species while no differences were found within species. Distance was also significantly higher ($p < 0.05$) when sequence information was considered (Figure 2.5).

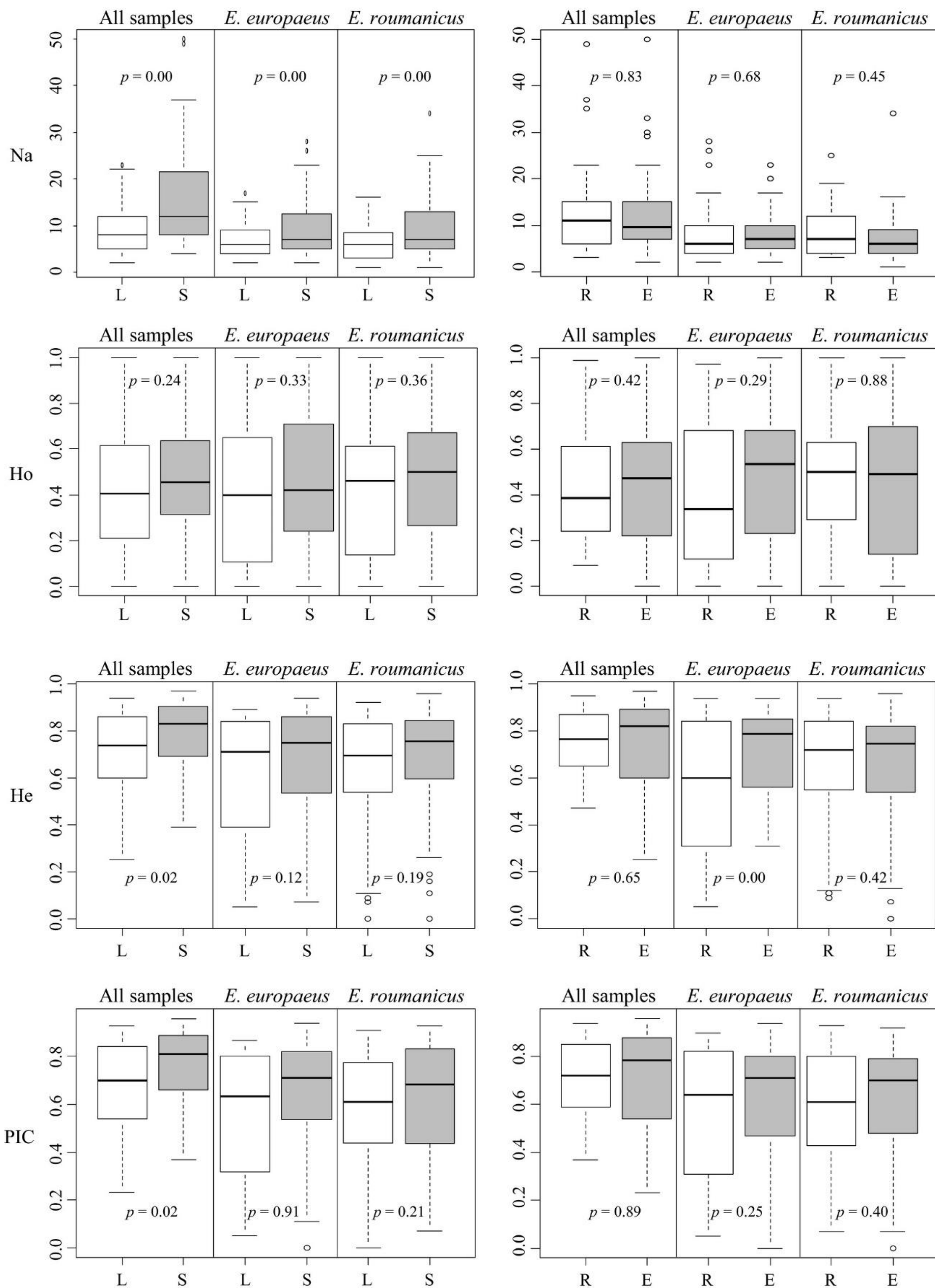


Figure 2.4: Boxplots describing variability and genetic diversity measurements per marker. Left panel using different allele calling approaches: sequence length (L) and sequence information (S). Right panel using different markers sets: *E. europaeus*-specific primers (E) and *E. roumanicus* species primers (R). *p*-Values correspond to *t*-tests comparing differences in averages between genotyping methods and markers sets

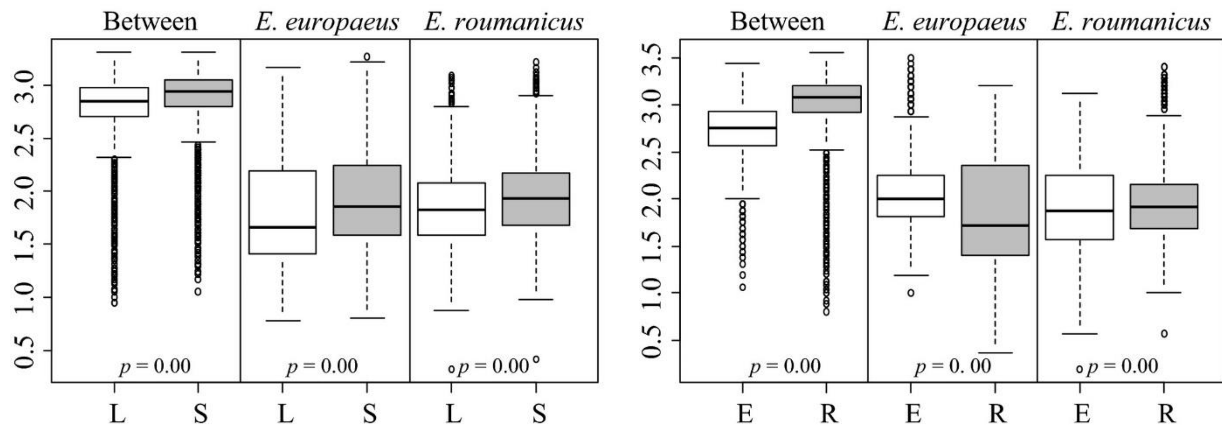


Figure 2.5: Boxplots describing pairwise distance between samples. Left panel using different allele calling approaches: sequence length (L) and sequence information (S). Right panel using different markers sets: *E. europaeus*-specific primers (E) and *E. roumanicus*

Genetic diversity and marker variability were not clearly different between the two marker sets used, although the set using markers specific for *E. europaeus* were slightly more diverse (Figure 2.4). This was only significant when only *E. europaeus* samples were used. When the same comparison was performed using genetic distance among individuals, one of the marker sets recovered significantly higher distances than the others (Figure 2.5), for all test. *E. roumanicus*-specific markers resulted in higher distances between species (Figure 2.5). Within species, *E. europaeus* markers contributed to a slightly higher distance among *E. europaeus* individuals. No difference between the marker sets is observed for *E. roumanicus* among the samples.

2.4.5 Genetic structure

When all individuals from both species were considered, the PCoA analysis resulted in two clear groups corresponding to the two species (Figure 2.6). There was one *E. roumanicus* individual from Linz (2016169) that appears in the *E. europaeus* group and one *E. europaeus* individual from east Linz (2014581) that groups together with the *E. roumanicus* samples. The PCoA also shows some samples that are in intermediate positions between both groups: one *E. europaeus* from Linz (2012159) and one *E. roumanicus* from the southern region of Linz (2016169). When considering only *E. europaeus* individuals, the PCoA showed three clear groups: one comprised by the samples collected by the Innsbruck shelter, another by the samples collected by the Vorarlberg shelter, and a last one containing the remaining samples. When considering only *E. roumanicus* individuals, two larger groups are found reflecting a separation between individuals from the northwestern and southeastern regions of the sampling: southeast being composed of the samples collected in the Klagenfurt shelter, Burgenland, Macedonia, Hungary, and Croatia; and

the northwest containing the remaining samples. Samples from the easternmost region of Austria (Neusiedlersee) seem to be between these two groups.

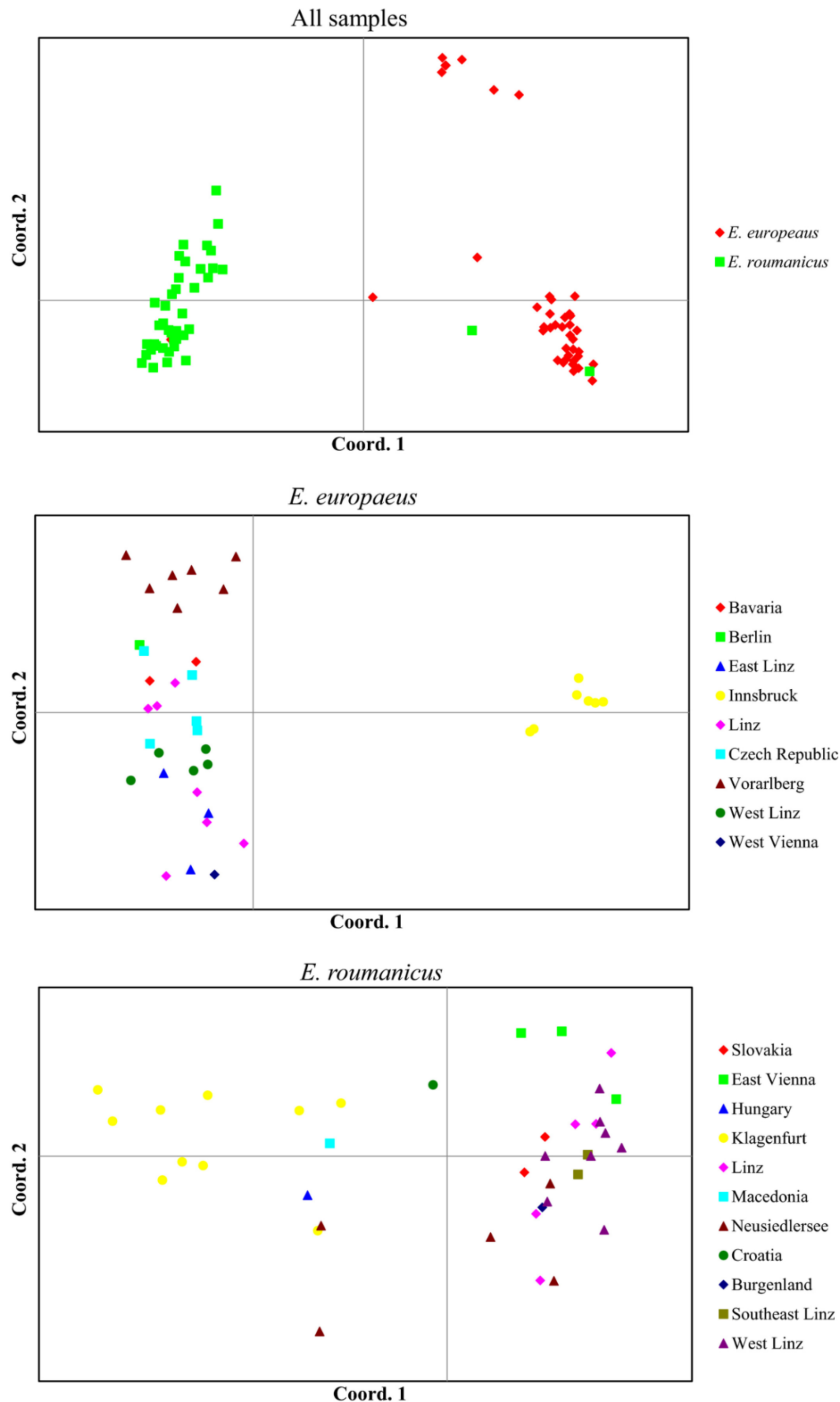


Figure 2.6: Principal coordinates analyses from matrix with genotypes called based on sequence information. Top: PCoA with complete dataset. Middle: PCoA with only *E. europaeus* samples. Bottom: PCoA with *E. roumanicus*. In the analysis for all samples, the samples; errata Error: *E. europeaus* Correction: *E. europaeus*

STRUCTURE analyses were congruent with the PCoA results. When both species were considered, the optimal K-value was two (Figure 2.7). For this analysis, both species were clearly separated into two clusters, with four samples showing either some degree of admixture or an opposite assignment to their morphological classification. These were the same individuals misidentified or showing signals of admixture in the PCoA analysis. The STRUCTURE analyses with only *E. europaeus* and *E. roumanicus* samples resulted in best K-values of 3 and 5, respectively. Nevertheless, we also considered lower values of K to see if there was any congruence between the hierarchy cluster divisions and geographical distribution. For *E. europaeus*, in the K = 2 analysis, samples from the Vorarlberg shelter and Berlin were separated from the remaining ones. For K = 3, the additional cluster contains only the individuals from the shelter in Innsbruck. Considering the *E. roumanicus* dataset, for K = 2, one of the clusters is more prevalent in southern Austria (Klagenfurt and Burgenland) and the other countries while the other in the west (Linz region). The localities geographically between these groups (Vienna and Neusidlersee) show some degree of admixture. This pattern corresponding to a gradual transition of a cluster from southeast to another in the northeast is congruent with a scenario of isolation by distance. For the higher values of K, the following subgroups are observed: for K = 3, samples from Vienna are separated from the rest; for K = 4, the shelter from Klagenfurt has its own cluster; and for K = 5, it is possible to observe a new cluster comprising some samples from Neusiedlersee, the sample from Burgenland, and one individual from West Linz. For both species, although significant, there was a small correlation between geographical and genetic distance (Supplementary Figure 2.1) indicating a slight signal of isolation by distance. This correlation was more pronounced for *E. europaeus* (r = 0.35) then *E. roumanicus* (r = 0.25).

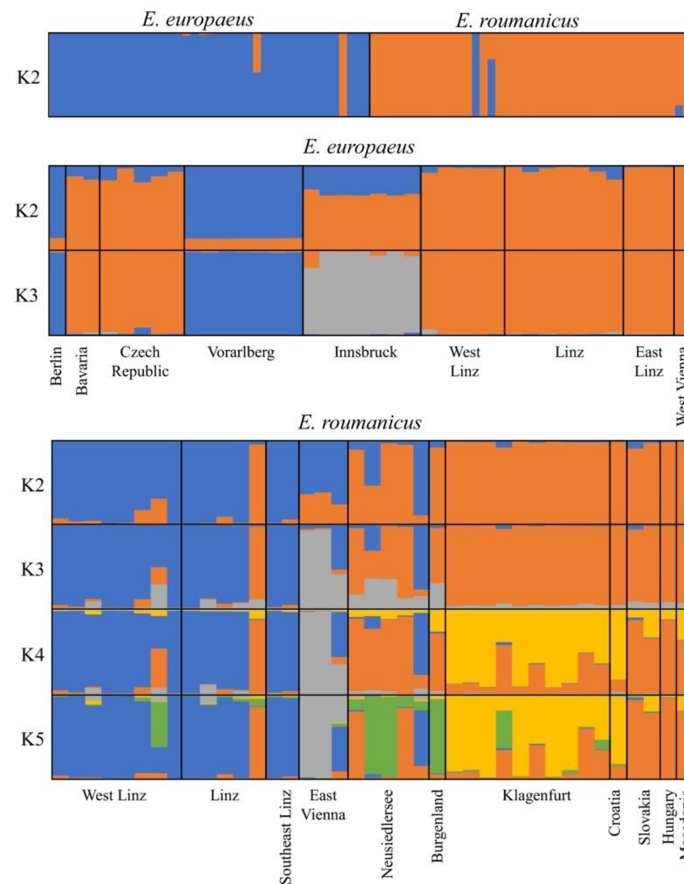


Figure 2.7: Structure analysis for all three datasets (All samples, only *E. europaeus*, only *E. roumanicus*) considering all markers and alleles called based on sequence information. Only results from K = 2 until the optimum are shown

Clustering results obtained with STRUCTURE, differed between the two allele calling approaches in particular for the *E. europaeus* dataset where the samples from Bavaria and Czech Republic had different assignments (Supplementary Figure 2.2). Overall, allele calling based on sequence information showed a lower number of individuals with mixed assignment. When the same analysis was used to test the impact of using species-specific primers, this resulted in a slightly clearer assignment for *E. europaeus* (Supplementary Figure 2.3), while for the *E. roumanicus* dataset the marker set played no role in recovering a clearer genetic structure pattern.

2.5 Discussion

In this study, we present a set of SSR markers that can be used for genotyping by sequencing of amplicons. The SSR-GBS approach provides a significant improvement over traditional fingerprinting methods, in particular because of three factors. First, laboratory methods are highly simplified, primarily due to the ability to utilize multiplexing PCR to a higher degree than when using fragment length analysis. Second, the ability to not only capture length polymorphisms but also SNPs results in more information for allele definition when compared to electrophoresis-based methods, resulting in higher resolution with the SSR-GBS approach. Third, the detection of alleles as sequences decreases ambiguity when allele calls are reproduced.

This facilitates the concatenation of existing with new data and the combination of different datasets. In the following sections, we review these potential improvements, beginning with the procedure details and concluding with a discussion of the prospects of compiling large datasets for genotype analyses in hedgehogs. In addition, although similar whole-genome genotyping without available reference sequences has been previously described (Andrews et al., 2016), we highlight the potential of the current method.

2.5.1 **Marker specificity**

In this study, we developed primers for two closely related species, which allows for the evaluation of cross-amplification capacity. We started with primers for *E. roumanicus,* because for this species until now no microsatellites had been developed, there exist marker sets for *E. europaeus*. When testing cross-species amplification, we not only found null alleles, as expected (Turini et al., 2014), but also discovered loci where the repeat unit was deleted in *E. europaeus* or invariable because the allele was fixed where the repeat motif was interrupted by a SNP, and thus, variability could no longer be measured. These markers gave a positive signal after amplification, but differ in evolutionary history and variability between and within species. This confirmed the need to develop additional markers for *E. europaeus*.

Marker selection based on their variation, their source material, and their amplification success in different species results in ascertainment bias (Brandström & Ellegren, 2008). It is common practice in microsatellite genotyping to maximize variability, and this may result in an overestimation of genetic diversity or high prevalence of null alleles (Ellegren, 2000; Huang et al., 2002; Weber & Wong, 1993). This leads to increased information content despite limited numbers of markers. When using GBS, the inclusion of additional markers does not increase the workload making this of less importance/unnecessary. Therefore, markers were not filtered based on variability. Developing marker sets based on several species minimizes ascertainment bias within each species. When biases related to the species of the marker set origin were evaluated, few differences were found in the results of genetic diversity; however, this was not the case for distance between samples and species. *E. roumanicus*-specific markers resulted in higher differentiation between species while those specific to *E. europaeus* resulted in a higher differentiation among individuals of the same species. This difference in performance between marker sets further indicate that using only one marker set could have contributed to the presence of variability biases in our dataset.

2.5.2 Better resolution

We showed that allele call considering complete sequence information (both length and SNPs) leads to higher values for marker variability, information content, and distance between species. This improvement was most likely related to a higher number of alleles recovered with sequence information. In most cases, sequence allele definition led to an increased number of alleles and PIC, which increased anywhere from zero to 267 % depending on the locus. Part of the improvement on the genetic structure may be due to the decrease in the amount of homoplasy, which is difficult to estimate with length polymorphism information alone. This was shown by the increase in singleton alleles when sequence information was used, which resulted in the division into multiple alleles of length polymorphism alleles with the same length but different nucleotide composition. However, the definition of alleles according to sequence information did not change much the overall structure assignment, likely as a consequence of the high number of markers used.

The decrease in homoplasy and the large number of markers can also explain the lack of significantly higher genetic diversity using the allele calling approach for some of the comparisons made. This was the case for H_O for all tests and for H_E and PIC at an intraspecific level. Homoplasy is more likely to be found when comparing both species, so it makes sense that the genetic diversity results were significantly higher when all samples were included but not necessarily within species or populations. Given the high number of markers, most of the variation was already recovered using the length approach. Within one population, individuals are more closely related; thus, it is less likely to find homoplasy. Consequently, with sequence-based genotyping we did not find a significantly higher genetic diversity at this level.

Studies using microsatellites on hedgehogs are currently based on two sets of markers that had been developed by Becher and Griffiths (1997) and by Henderson, Becher, Doncaster, and Maclean (2000) comprising a total of 11 loci. These markers were, in some cases, able to differentiate genetic clusters on a rather small spatial scale, which in other studies was not as pronounced (i.e., Braaker et al., 2017). For example, compared to Braaker et al. (2017), which found between 2 and 15 alleles with an average of 8, our study obtained a similar number of alleles while using only *E. europaeus* with species-specific markers and length information (between 2 and 17 with an average of 8). These numbers increased with sequence information, ranging between 3 and 23 with an average of 11.5. We included all markers showing an amplification product, despite possibly only being informative within one species, because they can be useful for intraspecific comparisons and other similar questions. For intraspecific

comparisons, we could concentrate on markers with high PIC and complement this with new loci. The high number of alleles found in some of our markers, for example, the markers W12 (50 alleles) and E23 (49 alleles), may be a consequence of gene duplication or scoring errors. Despite not finding an effect in the results, we recommend excluding them in further studies.

2.5.3 Simplification of the procedure

The laboratory methods are based on the amplicon sequencing approach suggested by Illumina and widely used for DNA bar coding (Cruaud, Rasplus, Rodriguez, & Cruaud, 2017; Shokralla et al., 2015). This approach allows a higher level of multiplexing than traditional methods, where typically up to four markers are combined in one PCR. This high number requires optimization which is only cost-effective in studies with a large number of samples. In the current experiments, we routinely multiplexed 10 markers; however, in one experiment up to 30 markers were successfully multiplexed in one reaction. In our previous work, based on the asymmetric PCR approach (Curto et al., 2015, 2013), we used a multiplex of four markers in an electrophoresis genotyping approach, with between 4 and 5 PCRs per sample and the same number of ABI electrophoreses. In comparison, with the system presented here, we can reach this amount with one or two PCRs and comparable primer costs.

2.5.4 Better reproducibility and easier analysis

The main advantage of using SSR-GBS is the better reproducibility of the data (de Barba et al., 2017). In traditional electrophoresis-based determinations of SSR alleles, mobility of DNA fragments in the polyacrylamide matrix (used in most applications) is measured against an internal dye-labeled size standard. The size of the allele is then called in comparison to the standard fragment sizes. The fragments do not always migrate through the capillary the same way, creating variation between runs, capillary sets, and laboratories (Davidson & Chiba, 2003; Fernando, Evans, Morales, & Melnick, 2001). In our experience, within one project different plates might differ by one or two base in size estimates, which requires manual control of the range within which each allele occurs. Using tetra- or pentanucleotide repeats, as frequently done with vertebrates, this is generally not a problem, but with di- and trinucleotides this effect is more problematic due to the length ranges of possible alleles (‘bins’) which are narrower for these motifs (Ginot, Bordelais, Nguyen, & Gyapay, 1996; Litt, Hauge, & Sharma, 1993). Additionally, *Taq* polymerase adds a single nucleotide to the 3' end of the PCR product, most frequently Adenine (Brownstein, Carpten, & Smith, 1996; Magnuson et al., 1996). As a frequent artefact which is observed depending on PCR performance, this cannot be omitted and an allele may be divided into two peaks that differ by one base. The so-called ‘plus A peak’ artefact is a

combination of this amplification artefact and variation of fragment and size standard migration in the electrical field. Ultimately, it can lead to errors of two to three base pairs, which can be further increased depending on the fluorescent dye used. The necessity for including samples of known genotype as a standard to verify allele identity is therefore common practice. As a result, the use of SNPs over SSR markers for high-interest species data collected by multiple laboratories has been suggested (e.g., for wolfs by Kraus et al., 2015).

In SSR-GBS, the 'plus A peak' artefact is no longer relevant as the allele definition is not dependent on positions upstream of the primer binding sites, and the ambiguity that stems from electrophoresis and the addition of extra bases by the enzyme is not applicable when the fragment length is determined by the sequence composition. However, slippage artefacts may still occur with SSR-GBS because of its' dependency on PCR and all of the optimization procedures (Ellegren, 2004). The method is, in this respect, comparable to electrophoresis-based methods, and therefore, ambiguities remain, especially for dinucleotide motifs.

Previous studies used primers already containing the index for sample identification and included only tetra- and pentanucleotide repeats to reduce PCR complexity and thus artefacts (de Barba et al., 2017). The high costs associated with this can be justified considering certain model systems such as *Ursus arctus*, a large carnivore with a high public interest, but not for small scale, non-model organism research, for which our method would be more appropriate. To gain experience of the method's properties, we decided to include dinucleotide repeats, which are frequently used in other systems, in particular for plants (Lagercrantz, Ellegren, & Andersson, 1993; Tóth, Gáspári, & Jurka, 2000). Dinucleotides, compared to tetra- and pentanucleotides, have a higher probability of producing stutter bands, which are problematic for allele determination (Ginot et al., 1996; Litt et al., 1993). Nevertheless, in most cases, this limitation can be overcome during the allele call procedure.

In the dataset presented here, allele calling was not performed completely automatically. De Barba et al. (2017) presented a pipeline for automated allele calling of sequence-based alleles (i.e., including SNPs). However, the procedure suggested did not work for dinucleotides, so a slightly different approach was chosen. First, we used the length polymorphisms to determine the SSR allele, that is the most likely allele definition according to length, and thus the repeat unit number. In a second step, we investigated whether the SSR allele contained additional single nucleotide polymorphisms or not. Similar to traditional electrophoresis-based analysis, this approach is very accurate for tetra- and pentanucleotide repeats, but has a higher error rate with

dinucleotides. Here, the difficulty in determining alleles when stutter bands of one allele overlay another still exists because the determination of the SSR allele is performed according to length frequency distribution and does not differ in this respect from traditional analyses. When both alleles differ on base composition, this overlay applies also to SNPs, which means that an SSR allele overlaid by a stutter band can show a nucleotide polymorphism as an artefact. Here, the state of the other allele must be taken into consideration. The approach of de Barba et al. (2017) is also unable to overcome this limitation since it divides alleles based on SNPs in the flaking regions first. The program HipSTR (Willems et al., 2017) can deal with the stutter effect by using a parametric approach. It defines candidate alleles based on a stutter model and uses them as reference to align the reads redefining new candidate alleles. This process is repeated until the most likely alignment is obtained. Since this approach is based on alignment quality, it is likely to be negatively affected by erroneous phasing between SNP variations in the flanking regions and the repetition motif. As mentioned above, this can be caused by the overlay of stutter bands and the formation of chimeric sequences in the PCR. These artefacts result in sequences containing the repetition motif of one allele and the SNP variant of the other. HipSTR does not have a filtering step where these error sources are considered, and thus, all sequences stemming from PCR artefacts are included during allele call. This can potentially contribute to a lower likelihood of alignments of the correct alleles. In our method, because we filter out reads first based on length, with a manual control step, a lot sources of error are already excluded, decreasing the ambiguity of the final allele calling. There are alternative approaches based on the assembly of the amplicon reads. Šarhanová, Pfanzelt, Brandt, Himmelbach, and Blattner (2018) applied an alternative approach based on read de novo assembly. Nevertheless, a manual control step was added to account for the assembly of two alleles filtering noise. Thus, at this moment, a manual curation step is still necessary in the genotyping of di- and trinucleotides repeats.

The high reproducibility that can be achieved in determining sequence alleles also allows for the easy creation of large data collections over multiple laboratories and projects. There are several examples where SSR variation is used for wildlife monitoring; however, the technical difficulties restrict this to species for which there is considerable conservation concern (Godinho et al., 2011), conflict species (De Barba et al., 2010), or species with large commercial interest (Schenekar & Weiss, 2017; Tibihika, Waidbacher et al., 2018). With similar approaches to the SSR-GBS system, this can be adapted for non-model species and specific scientific questions. Our interest in hedgehogs resulted from a citizen science project, where occurrence data had been collected in private gardens together with that from primary school students and the general public. The prospect of including methods that allow for investigation of a variety of

samples, using hair, faeces, or mouth swabs is very interesting and could be achieved by the SSR-GBS system presented here. In our case, although mouth swabs showed higher missing data than tissue samples this did not affect the final results. This was a consequence of a lower number of reads for these samples. The potential of SSR-GBS can be compared to phylogenetic data collections, where sequences can very easily be incorporated into existing alignments and large meta-analyses are frequent (Adams, 2008). It therefore constitutes a tool that can be implemented in long-term screening projects.

2.5.5 Phylogeographic implications

Two of the included individuals were detected as potential hybrids. Using a dense sampling from the contact zone in the Check Republic, Bolfíková and Hulva (2012) did not find any evidence of hybridization among the two hedgehog species. However, hybridization among these species would be congruent with the high incidence of hybrid zones in central Europe (Hewitt, 2001). The current rarity of hybridization events can be a remnant of a hybrid zone dynamics. It is likely that every time these species contacted after a glacial period a hybrid zone was established. With time, these species may have become more reproductively isolated to a point that the hybrid zone either only exists in some areas or it is very narrow. This hypothesis can only be tested by characterizing hybridization occurrence and frequency across the contact zone.

Overall there was a weak correlation between genetic structure and geographical distance, which may be a consequence of barrier to gene flow, promoted by natural and anthropogenic factors. For example, there was a separation among *E. roumanicus* individuals from the south and north of the alps indicating that these mountains may work as a natural barrier. Additionally, human structures such as roads may have contributed to some structure found at the local level (Braaker et al., 2017). This has been reported to be the case for *E. europaeus* populations in England (Becher and Griffiths 1998). The potential role of natural anthropogenic structures on hedgehog populations from central Europe needs to be better investigated with a denser sampling in order to account for small scale genetic structure as well.

Shelters' practices may also influence the distribution of genetic variability. This happens when the source of the individuals are unknown and they are consequently not released in areas of their origin. This may contribute to outbreeding depression and promote hybridization (Edmands, 2007). In the current study, the individuals from the shelters are genetically homogeneous, so as long as the shelter does not release individuals outside the area of activity the gene pool of natural populations should not be affected. Given the low amount of shelters

and limited sampling, it is still impossible to make any conclusion in this matter and we are currently in the process of including a larger sampling from multiple shelters spread throughout Central Europe.

2.5.6 Importance of the museum collections

The improvement of replicability associated with the SSR-GBS approach may allow several long-term studies using newly collected and museum samples. For our study, we were able to utilize a large collection of hedgehog specimens preserved in ethanol at the Biologiezentrum in Linz. This emphasizes the usefulness of the storage of multiple samples, especially from species that attract public attention, by public collections. In the Biologiezentrum Linz, this was achieved by combining several private collections with staff efforts, from which studies like this one benefit. This also demonstrates how desirable it is to store multiple samples per species even if space problems and considerations of general funds might suggest otherwise. This is especially true when, like in the present dataset, potential hybrids are found and the determination of morphological characters may be critical to complement the molecular data.

2.6 Acknowledgements

The authors thank the Biologiezentrum Linz, the Natural History Museum in Vienna, the Leibniz Institute for Zoo and Wildlife Research and the Museum in Linz for providing samples. The shelters Bludenz, Innsbruck, Klagenfurt are acknowledged for their help to collect salvia samples. Eva Dornstauder-Schramler provided technical assistance. The research was partly funded by the University of Natural Resources and Life Sciences, Vienna through the project 'MICSATGEN' attributed to Silvia Winter.

2.7 References

1000 Genomes Project Consortium (2010). A map of human genome variation from population-scale sequencing. Nature, 467(7319), 1061– 1073. https://doi.org/10.1038/nature09534

Adams, D. C. (2008). Phylogenetic meta-analysis. Evolution, 62(3), 567– 572. https://doi.org/10.1111/j.1558-5646.2007.00314.x

Andolfatto, P., Davison, D., Erezyilmaz, D., Hu, T. T., Mast, J., Sunayama-Morita, T., & Stern, D. L. (2011). Multiplexed shotgun genotyping for rapid and efficient genetic mapping. Genome Research, 21, 610– 617. https://doi.org/10.1101/gr.115402.110

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics, 17(2), 81– 92. https://doi.org/10.1038/nrg.2015.28

Arnold, B., Corbett-Detig, R. B., Hartl, D., & Bomblies, K. (2013). RADseq underestimates diversity and

introduces genealogical biases due to nonrandom haplotype sampling. Molecular Ecology, 22(11), 3179– 3190. https://doi.org/10.1111/mec.12276

Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., … Johnson, E. A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE, 3(10), e3376.

Becher, S. A., & Griffiths, R. (1997). Isolation and characterization of six polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus*. Molecular Ecology, 6(1), 89– 90.

Becher, S. A., & Griffiths, R. (1998). Genetic differentiation among local populations of the European hedgehog (*Erinaceus europaeus*) in mosaic habitats. Molecular Ecology, 7(11), 1599– 1604.

Bogdanov, A. S., Bannikova, A. A., Pirusskii, Y. M., & Formozov, N. A. (2009). The first genetic evidence of hybridization between West European and Northern white-breasted hedgehogs (*Erinaceus europaeus* and *E. roumanicus*) in Moscow region. Biology Bulletin, 36(6), 647. https://doi.org/10.1134/S106235900906017X

Bolfíková, B. Č., Eliášová, K., Loudová, M., Kryštufek, B., Lymberakis, P., Sándor, A. D., & Hulva, P. (2017). Glacial allopatry vs. postglacial parapatry and peripatry: The case of hedgehogs. PeerJ, 5, e3163.

Bolfíková, B., & Hulva, P. (2012). Microevolution of sympatry: Landscape genetics of hedgehogs *Erinaceus europaeus* and *E. roumanicus* in Central Europe. Heredity, 108(3), 248.

Braaker, S., Kormann, U., Bontadina, F., & Obrist, M. K. (2017). Prediction of genetic connectivity in urban ecosystems by combining detailed movement data, genetic data and multi-path modelling. Landscape and Urban Planning, 160, 107– 114. https://doi.org/10.1016/j.landurbplan.2016.12.011

Brandström, M., & Ellegren, H. (2008). Genome-wide analysis of microsatellite polymorphism in chicken circumventing the ascertainment bias. GenomeResearch, 8, 881– 887. https://doi.org/10.1101/gr.075242.107

Brownstein, M. J., Carpten, J. D., & Smith, J. R. (1996). Modulation of non-templated nucleotide addition by Taq DNA polymerase: Primer modifications that facilitate genotyping. BioTechniques, 20, 1004– 1010.

Callen, D. F., Thompson, A. D., Shen, Y., Phillips, H. A., Richards, R. I., Mulley, J. C., & Sutherland, G. R. (1993). Incidence and origin of' null' alleles in the (AC) n microsatellite markers. American Journal of Human Genetics, 52(5), 922.

Campbell, N. R., Harmon, S. A., & Narum, S. R. (2015). Genotyping-in-Thousands by sequencing (GT-seq): A cost effective SNP genotyping method based on custom amplicon sequencing. Molecular Ecology Resources, 15(4), 855– 867. https://doi.org/10.1111/1755-0998.12357

Corander, J., & Marttinen, P. (2006). Bayesian identification of admixture events using multilocus molecular markers. Molecular Ecology, 15(10), 2833– 2843. https://doi.org/10.1111/j.1365-294X.2006.02994.x

Cronn, R., Knaus, B. J., Liston, A., Maughan, P. J., Parks, M., Syring, J. V., & Udall, J. (2012). Targeted

enrichment strategies for next-generation plant biology. American Journal of Botany, 99, 291– 311.

Cruaud, P., Rasplus, J. Y., Rodriguez, L. J., & Cruaud, A. (2017). High-throughput sequencing of multiple amplicons for barcoding and integrative taxonomy. Scientific Reports, 7, 41948. https://doi.org/10.1038/srep41948

Curto, M., Nogueira, M., Beja, P., Amorim, F., Schümann, M., & Meimberg, H. (2015). Influence of past agricultural fragmentation to the genetic structure of Juniperus oxycedrus in a Mediterranean landscape. Tree Genetics & Genomes, 11(2), 32. https://doi.org/10.1007/s11295-015-0861-2

Curto, M., Schachtler, C., Puppo, P., & Meimberg, H. (2018). Using a new RAD-sequencing approach to study the evolution of Micromeria in the Canary islands. Molecular Phylogenetics and Evolution, 119, 160– 169. https://doi.org/10.1016/j.ympev.2017.11.005

Curto, M. A., Tembrock, L. R., Puppo, P., Nogueira, M., Simmons, M. P., & Meimberg, H. (2013). Evaluation of microsatellites of Catha edulis (qat; Celastraceae) identified using pyrosequencing. Biochemical Systematics and Ecology, 49, 1– 9. https://doi.org/10.1016/j.bse.2013.02.002

Daetwyler, H. D., Capitan, A., Pausch, H., Stothard, P., Van Binsbergen, R., Brøndum, R. F., … Esquerré, D. (2014). Whole-genome sequencing of 234 bulls facilitates mapping of monogenic and complex traits in cattle. NatureGenetics, 46(8), 858– 865. https://doi.org/10.1038/ng.3034

Davidson, A., & Chiba, S. (2003). Laboratory temperature variation is a previously unrecognised source of genotyping error during capillary electrophoresis. Molecular Ecology Notes, 3, 321– 323.

De Barba, M., Miquel, C., Lobréaux, S., Quenette, P. Y., Swenson, J. E., & Taberlet, P. (2017). High-throughput microsatellite genotyping in ecology: Improved accuracy, efficiency, standardization and success with low-quantity and degraded DNA. Molecular Ecology Resources, 17(3), 492– 507. https://doi.org/10.1111/1755-0998.12594

De Barba, M., Waits, L. P., Garton, E. O., Genovesi, P., Randi, E., Mustoni, A., & Groff, C. (2010). The power of genetic monitoring for studying demography, ecology and genetics of a reintroduced brown bear population. Molecular Ecology, 19, 3938– 3951. https://doi.org/10.1111/j.1365-294X.2010.04791.x

Earl, D. A. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation genetics resources, 4(2), 359– 361.

Edmands, S. (2007). Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology, 16(3), 463– 475. https://doi.org/10.1111/j.1365-294X.2006.03148.x

Ellegren, H. (2000). Heterogeneous mutation processes in human microsatellite DNA sequences. Nature Genetics, 24, 400– 402.

Ellegren, H. (2004). Microsatellites: Sample sequences with complex evolution. Nature Reviews Genetics, 5, 435– 445.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS

one, 6(5), e19379.

Farrell, E. D., Carlsson, J. E., & Carlsson, J. (2016). Next Gen Pop Gen: Implementing a high-throughput approach to population genetics in boarfish (Capros aper). Open Science, 3(12), 160651.

Fernando, P., Evans, B. J., Morales, J. C., & Melnick, D. J. (2001). Electrophoresis artefacts — A previously unrecognised cause of error in microsatellite analysis. Molecular Ecology Notes, 1, 325–328.

Ginot, F., Bordelais, I., Nguyen, S., & Gyapay, G. (1996). Correction of some genotyping errors in automated fluorescent microsatellite analysis by enzymatic removal of one base overhangs. Nucleic Acids Research, 24, 540– 541. https://doi.org/10.1093/nar/24.3.540

Godinho, R., Llaneza, L., Blanco, J. C., Lopes, S., Alvares, F., Garcia, E. J., … Ferrand, N. (2011). Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. Molecular Ecology, 20(24), 5154– 5166. https://doi.org/10.1111/j.1365-294X.2011.05345.x

Hardenbol, P., Banér, J., Jain, M., Nilsson, M., Namsaraev, E. A., Karlin-Neumann, G. A., … Davis, R. W. (2003). Multiplexed genotyping with sequence-tagged molecular inversion probes. NatureBiotechnology, 21(6), 673. https://doi.org/10.1038/nbt821

Henderson, M., Becher, S. A., Doncaster, C. P., & Maclean, N. (2000). Five new polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus*. Molecular Ecology, 9(11), 1949–1951.

Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society, 68(1–2), 87– 112. https://doi.org/10.1111/j.1095-8312.1999.tb01160.x

Hewitt, G. M. (2001). Speciation, hybrid zones and phylogeography—Or seeing genes in space and time. Molecular Ecology, 10(3), 537– 549. https://doi.org/10.1046/j.1365-294x.2001.01202.x

Hodel, R. G., Chen, S., Payton, A. C., McDaniel, S. F., Soltis, P., & Soltis, D. E. (2017). Adding loci improves phylogeographic resolution in red mangroves despite increased missing data: Comparing microsatellites and RAD-Seq and investigating loci filtering. Scientific Reports, 7, 17598. https://doi.org/10.1038/s41598-017-16810-7

Hodel, R. G., Segovia-Salcedo, M. C., Landis, J. B., Crowl, A. A., Sun, M., Liu, X., … Soltis, D. E. (2016). The report of my death was an exaggeration: A review for researchers using microsatellites in the 21st century. Applications in Plant Sciences, 4(6), 1600025. https://doi.org/10.3732/apps.1600025

Huang, Q. Y., Xu, F. H., Shen, H., Deng, H. Y., Liu, Y. J., Liu, Y. Z., … Deng, H. W. (2002). Mutation patterns at dinucleotide microsatellite loci in humans. American Journal of Human Genetics, 70, 625– 634.

Hubisz, M. J., Falush, D., Stephens, M., & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources, 9(5), 1322– 1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Huijser, M. P., & Bergers, P. J. (2000). The effect of roads and traffic on hedgehog (*Erinaceus europaeus*) populations. Biological Conservation, 95(1), 111– 116. https://doi.org/10.1016/S0006-3207(00)00006-9

Johansson, S. A., Karlsson, P., & Gyllensten, U. (2003). A novel method for automatic genotyping of microsatellite markers based on parametric pattern recognition. Human Genetics, 113(4), 316– 324.

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology, 16(5), 1099– 1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Thierer, T. (2012). Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647– 1649. https://doi.org/10.1093/bioinformatics/bts199

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Molecular ecology resources, 15(5), 1179– 1191.

Kraus, R. H., Vonholdt, B., Cocchiararo, B., Harms, V., Bayerl, H., Kühn, R., … Nowak, C. (2015). A single-nucleotide polymorphism-based approach for rapid and cost-effective genetic wolf monitoring in Europe based on noninvasively collected samples. Molecular Ecology Resources, 15(2), 295– 305. https://doi.org/10.1111/1755-0998.12307

Lagercrantz, U., Ellegren, H., & Andersson, L. (1993). The abundance of various polymorphic microsatellite motifs differs between plants and vertebrates. Nucleic Acids Research, 21(5), 1111– 1115. https://doi.org/10.1093/nar/21.5.1111

Lemmon, A. R., Emme, S. A., & Lemmon, E. M. (2012). Anchored hybrid enrichment for massively high-throughput phylogenomics. Systematic Biology, 61(5), 727– 744. https://doi.org/10.1093/sysbio/sys049

Li, H., & Durbin, R. (2011). Inference of human population history from individual whole-genome sequences. Nature, 475(7357), 493– 496.

Litt, M., Hauge, X., & Sharma, V. (1993). Shadow bands seen when typing polymorphic dinucleotide repeats — Some causes and cures. BioTechniques, 15, 280– 284 et seq.

Magnuson, V. L., Ally, D. S., Nylund, S. J., Karanjawala, Z. E., Rayman, J. B., Knapp, J. I., … Collins, F. S. (1996). Substrate nucleotide-determined non-templates addition of adenine by Taq DNA polymerase: Implications for PCR-based genotyping and cloning. BioTechniques, 21, 700– 709.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. Embnet.journal, 17(1), 10. https://doi.org/10.14806/ej.17.1.200

Meimberg, H., Hammond, J. I., Jorgensen, C. M., Park, T. W., Gerlach, J. D., Rice, K. J., & McKay, J. K. (2006). Molecular evidence for an extreme genetic bottleneck during introduction of an invading grass to California. Biological Invasions, 8(6), 1355– 1366.

Miller, M. P., Knaus, B. J., Mullins, T. D., & Haig, S. M. (2013). SSR_pipeline: A bioinformatic infrastructure for identifying microsatellites from paired-end Illumina high-throughput DNA sequencing data. Journal of Heredity, 104(6), 881– 885. https://doi.org/10.1093/jhered/est056

Orlowski, G., & Nowak, L. (2004). Road mortality of hedgehogs *Erinaceus spp.* in farmland in Lower Silesia (south-western Poland). Polish Journal of Ecology, 52(3), 377– 382.

Peakall, R. O. D., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Resources, 6(1), 288– 295.

R Core Team (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Rubin, C. J., Zody, M. C., Eriksson, J., Meadows, J. R., Sherwood, E., Webster, M. T., … Hallböök, F. (2010). Whole-genome resequencing reveals loci under selection during chicken domestication. Nature, 464(7288), 587– 591.

Ryman, N., Palm, S., André, C., Carvalho, G. R., Dahlgren, T. G., Jorde, P. E., … Ruzzante, D. E. (2006). Power for detecting genetic divergence: Differences between statistical methods and marker loci. Molecular Ecology, 15(8), 2031– 2045. https://doi.org/10.1111/j.1365-294X.2006.02839.x

Santucci, F., Emerson, B. C., & Hewitt, G. M. (1998). Mitochondrial DNA phylogeography of European hedgehogs. Molecular Ecology, 7(9), 1163– 1172. https://doi.org/10.1046/j.1365-294x.1998.00436.x

Šarhanová, P., Pfanzelt, S., Brandt, R., Himmelbach, A., & Blattner, F. R. (2018). SSR-seq: Genotyping of microsatellites using next-generation sequencing reveals higher level of polymorphism as compared to traditional fragment size scoring. Ecology and Evolution, 8, 10817– 10833. https://doi.org/10.1002/ece3.4533

Schenekar, T., & Weiss, S. (2017). Selection and genetic drift in captive versus wild populations: An assessment of neutral and adaptive (MHC-linked) genetic variation in wild and hatchery brown trout (Salmo trutta) populations. Conservation Genetics, 1– 12. https://doi.org/10.1007/s10592-017-0949-3

Schlotterer, C. (2000). Evolutionary dynamics of microsatellite DNA. Chromosoma, 109, 365– 371. https://doi.org/10.1007/s004120000089

Schopen, G. C. B., Bovenhuis, H., Visker, M. H. P. W., & Arendonk, J. A. M. (2008). Comparison of information content for microsatellites and SNPs in poultry and cattle. Animal genetics, 39(4), 451– 453.

Seddon, J. M., Santucci, F., Reeve, N. J., & Hewitt, G. M. (2001). DNA footprints of European hedgehogs, *Erinaceus europaeus* and *E. concolor*: Pleistocene refugia, postglacial expansion and colonization routes. Molecular Ecology, 10(9), 2187– 2198.

Shokralla, S., Porter, T. M., Gibson, J. F., Dobosz, R., Janzen, D. H., Hallwachs, W., … Hajibabaei, M. (2015). Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. Scientific Reports, 5, 9687. https://doi.org/10.1038/srep09687

Smith, A. M., Heisler, L. E., St. Onge, R. P., Farias-Hesson, E., Wallace, I. M., Bodeau, J., … Nislow, C. (2010). Highly-multiplexed barcode sequencing: An efficient method for parallel analysis of pooled samples. Nucleic Acids Research, 38(13), e142– e142. https://doi.org/10.1093/nar/gkq368

Sonah, H., Bastien, M., Iquira, E., Tardivel, A., Légaré, G., Boyle, B., … Belzile, F. (2013). An improved genotyping by sequencing (GBS) approach offering increased versatility and efficiency of SNP discovery and genotyping. PLoS ONE, 8(1), e54603. https://doi.org/10.1371/journal.pone.0054603

Tibihika, P. D., Curto, M., Dornstauder-Schrammel, E., Winter, S., Alemayehu, E., Waidbacher, H., & Meimberg, H. (2018). Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African Oreochromis niloticus. Conservation Genetics, Online First. https://doi.org/10.1007/s10592-018-1136-x

Tibihika, P. D., Waidbacher, H., Masembe, C., Curto, M., Sabatino, S., Alemayehu, E., … Meimberg, H. (2018). Anthropogenic impacts on the contextual morphological diversification and adaptation of Nile tilapia (Oreochromis niloticus, L. 1758) in East Africa. Environmental Biology of Fishes, 101, 363– 381.

Tóth, G., Gáspári, Z., & Jurka, J. (2000). Microsatellites in different eukaryotic genomes: Survey and analysis. GenomeResearch, 10(7), 967– 981. https://doi.org/10.1101/gr.10.7.967

Turini, F. G., Steinert, C., Heubl, G., Bringmann, G., Lombe, B. K., Mudogo, V., & Meimberg, H. (2014). Microsatellites facilitate species delimitation in Congolese Ancistrocladus (Ancistrocladaceae), a genus with pharmacologically potent naphthylisoquinoline alkaloids. Taxon, 63, 329– 341.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. Nucleic Acids Research, 40(15), e115– e115. https://doi.org/10.1093/nar/gks596

Varshney, R. K., Graner, A., & Sorrells, M. E. (2005). Genic microsatellite markers in plants: Features and applications. Trends in Biotechnology, 23(1), 48– 55. https://doi.org/10.1016/j.tibtech.2004.11.005

Vartia, S., Villanueva-Cañas, J. L., Finarelli, J., Farrell, E. D., Collins, P. C., Hughes, G. M., … FitzGerald, R. D. (2016). A novel method of microsatellite genotyping-by-sequencing using individual combinatorial barcoding. Royal Society Open Science, 3(1), 150565. https://doi.org/10.1098/rsos.150565

Weber, J. L., & Wong, C. (1993). Mutation of human short tandem repeats. Human Molecular Genetics, 2, 1123– 1128.

Willems, T., Zielinski, D., Yuan, J., Gordon, A., Gymrek, M., & Erlich, Y. (2017). Genome-wide profiling of heritable and de novo STR variations. NatureMethods, 14(6), 590. https://doi.org/10.1038/nmeth.4267

Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2013). PEAR: A fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics, 30(5), 614– 620.

https://doi.org/10.1093/bioinformatics/btt593

3 An easy, flexible solution to attach devices to hedgehogs (*Erinaceus europaeus*) enables long-term high resolution studies

published in Ecology and Evolution

Barthel, LMF, Hofer, H, Berger, A. An easy, flexible solution to attach devices to hedgehogs (*Erinaceus europaeus*) enables long-term high-resolution studies. *Ecol Evol*. 2019; 9: 672– 679. https://doi.org/10.1002/ece3.4794

License: Attribution 4.0 International (CC BY 4.0)

Leibniz Institute for Zoo and Wildlife Research (IZW), Department of Evolutionary Ecology
Alfred- Kowalke-Str. 17, D- 10315 Berlin, Germany
Berlin-Brandenburg Institute of Advanced Biodiversity Research, Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany
corresponding author: Barthel@izw-berlin.de, ++49+30-5168327

3.1 Abstract

1. Bio-logging is an essential tool for the investigation of behaviour, ecology and physiology of wildlife. This burgeoning field enables the improvement of population monitoring and conservation efforts, particularly for small, elusive animals where data collection is difficult. Device attachment usually requires species-specific solutions to ensure that data loggers exert minimal influence on the animal's behaviour and physiology, and ensure high reliability of data capture. External features or peculiar body shapes often make securing devices difficult for long-term monitoring, as in the case with small spiny mammals.
2. Here, we present a method that enables high-resolution, long-term investigations of European hedgehogs (*Erinaceus europaeus*) via GPS and acceleration loggers. We collected data from 17 wild hedgehogs with devices attached between 9 and 42 days.
3. Our results showed that hedgehogs behaved naturally; as individuals curled-up, moved through dense vegetation, slipped under fences and built regular day nests without any indication of impediment.
4. Our novel method makes it possible to not only attach high-precision devices for substantially longer than previous efforts, but enables detachment and reattachment of devices to the same individual. This makes it possible to quickly respond to unforeseen events and exchange devices, and overcomes the issue of short battery life common to many lightweight loggers.

Keywords: accelerometer, attachment, biotelemetry, device, *Erinaceus*, GPS, long-term measurement

3.2 Introduction

A thorough knowledge of the ecology, behaviour and physiology of species under free-ranging conditions is essential to understand their environmental needs, life-history strategies and thus is the crucial basis for their protection and conservation (Wilson & McMahon, 2006; Kays, Crofoot, Jetz, & Wikelski, 2015; LaPoint, Balkenhol, Hale, Sadler, & van der Ree, 2015). Research on movement patterns, habitat use, interspecies and intraspecies interactions, foraging and reproductive behaviour is essential for effective conservation management (Graham, Douglas-Hamilton, Adams, & Lee, 2009, Fraser et al. 2018). Such research, benefits from high resolution, long term data collection and can help develop effective nature reserves (Afonso, Fontes, Holland, & Santos, 2008), solve human-wildlife conflicts (Voigt et al., 2014) and improve captive breeding to ensure successful re-introduction of endangered species (Kaczensky et al., 2011). This field of 'big-data animal tracking' is advancing with the development of lightweight bio-logging devices capable of combining accelerometer, VHF and/or GPS (Kays et al. 2015).

The results of studies on behaviour and physiology of wildlife under controlled conditions can often not be reproduced under natural conditions, making studies on free-ranging animals in their native habitat crucial (Gattermann et al., 2008). Unfortunately, field studies can be difficult as observer presence is known to influence animal behaviour; however subtle the observation conditions might be (Schneirla, 1950; Scheibe & Gromann, 2006; Cagnacci, Boitani, Powell, & Boyce, 2010; Crofoot, Lambert, Kays, & Wikelski, 2010; Shamoun-Baranes et al., 2012; Kays et al., 2015). Innovative bio-telemetry/bio-logging technologies are being applied to an ever-increasing range of taxa (from insects to mammals), spatial scales (from habitat patch to continental scale), and habitats (from coral reefs to rainforests) enabling us to gain a deeper insight into the natural behaviour and physiology of species without the need for observer presence (Wilson & McMahon, 2006; Cagnacci et al., 2010; Kays et al., 2015). Yet, these devices may also change the behaviour of the animals to which they are attached or may influence their chance of survival, thereby also biasing results (e.g. Hofer & East, 1998). In order to reduce such biases, the data logger, attachment and handling procedure should all minimise disturbance of the study animals (Hofer & East, 1998; Pearl, 2000; Barron, Brawn, & Weatherhead, 2010; Collins, Petersen, Carr, & Pielstick, 2014; S. P. Vandenabeele et al., 2015). Therefore devices should be designed in a species-specific manner. Such a design needs to take into account the mass, size, shape and material of the device and the method of its attachment and potential for

detachment/reattachment (Culik, Bannasch, & Wilson, 1994; Bridge et al., 2011; Vandenabeele, Wilson, & Wikelski, 2013; Kays et al., 2015).

Concerning the mass of the device, it is recommended that a complete radio transmitter should not exceed 2–5 % of body mass (Hofer & East, 1998; Kenward, 2001; Sikes, Gannon, & Animal Care and Use Committee, 2011). Despite the wide acceptance of the 'percentage rule', a meta-analysis of bird behavioural studies found little evidence that the impact of carrying the device was proportional to its weight (Barron et al., 2010). In contrast, in a study of equids, Brooks, Bonyongo and Harris (2008) showed that, even within the accepted norms, small differences in collar mass can significantly affect specific behaviours. Regardless, both studies found that attachment position and collar fit impacted behaviours significantly (Brooks, Bonyongo and Harris, 2008; Barron et al., 2010). A key issue is that battery mass and size are the driving factors of device total mass. Together they determine battery life and thus the duration of data collection. The trade-off between light mass and long duration is particularly challenging when the study species are small, such as many lizards, birds or small mammals (Warner, Thomas, & Shine, 2006; Warwick, Morris, & Walker, 2006; Doody, Roe, Mayes, & Ishiyama, 2009; Flesch, Duncan, Pascoe, & Mulley, 2009; Dervo et al., 2010; Rautio, 2015).

While battery life and device size pose substantial hurdles for study design, data retrieval is by far the most important aspect of any study. The current methods for device attachment dictate that devices fall off upon glue deterioration and/or after the growth of fur or feathers or the shedding of spines. Yet this approach may not always be viable and animal recapture and manual device removal is also commonly necessary. As such, the swift and easy removal and reattachment of devices enables data download, battery exchange and the potential for prolongation of data collection. While solutions to battery life via solar-powered devices have enabled long term data collection in diurnal species, nocturnal animals are still considered elusive and bio-logging study design must be approached differently.

European hedgehogs (*Erinaceus europaeus*) are a small, nocturnal mammal (ranging seasonally from 600 to 1500g) with a highly flexible body covered in spines. These spines are made of keratin and are repeatedly shed during a hedgehog's lifetime. Individuals hide and forage in dense vegetation and have a number of interesting behaviours such as self-anointing, curling up, hibernation and regular nest building (Reeve, 1994; Hof, 2009; Reeve, Bowen, & Gurnell, 2015). Because of these characteristics and their unusual body shape, standard collars cannot be used and other methods of attachment are often unsuitable. For a more comprehensive understanding

of hedgehog behaviour longer-term data sets are extremely important and contingent on appropriate device selection and attachment.

Here we present a modified method of device attachment to hedgehogs that does not hinder the animal's movements and can be easily removed and replaced, thereby solving the trade-off between small device size and the collection of long-term high-resolution data.

3.3 Methods

3.3.1 Study area & animals

Fieldwork was conducted from August to September 2016 in a study area of 16 ha within an urban park of 88.2 ha, in southeast Berlin, Germany (52.48846°N, 13.46974°E) as part of an ongoing project. The park is open to the general public and comprises short grass, variable shrub density, gravel foot paths, a playground and a monument site. The park is surrounded by urban pedestrian areas, tarmacked streets and parking areas to the east and south, and is bounded by the river Spree to the north and by a railway embankment to the west. The park was open to the general public throughout day and night.

When traversing this urban park, hedgehogs may have to cross streets, slip through fences or climb up a railway embankment. In preparation of this study, three night surveys to find hedgehogs were carried out at least one hour after sunset to find the animals by spotlighting (P14.2, LED Lenser, Solingen, Germany). Every hedgehog was marked with five labelled shrink tubes on the spines (Mori et al., 2015). The tubes were labelled with a number starting with 1 to make it possible to identify them during recapture (N. J. Reeve, pers. comm. 2016).

3.3.2 Backpack attachment

The complete backpack comprised three components: the back plate, the data logger (GPS and accelerometer) and a very high frequency (VHF) transmitter (Figure 3.1 A).

The back plate consisted of 2.5 cm wide and 1.6 mm thick fabric material, a synthetic woven material made from polyethylene often used for belts, cut into 4.5 cm long strips. Four holes were burned into this fabric using a soldering iron to facilitate entry of two wires of different length (7.5 cm and 10 cm) (Figure 3.1 B). These wires were later used to fasten the devices (datalogger and transmitter) to the back plate (Figure 3.1 B) and could be used to easily attach or remove different devices to and from the plate. Some of the VHF transmitters had small tubes attached to them; therefore it was easy to just insert the wires. Others had to be glued to a different spot directly between the spines. For fixation wires were twisted, trimmed to length and

bent in such a way that they were aligned with the devices to prevent entangling or poking the hedgehogs. We tested different wires (steel, insulated copper, florist's wire) of which the insulated copper wire with a 1 mm diameter turned out to be best as it lasted longer. After inserting the wires through the fabric from below, a piece of soft Velcro (loop strap, 2.5 x 4.5 cm in size) was glued to the lower surface of the fabric, thereby fixing the wires in place and maximising the surface available for the attachment of the complete backpack to the animal. The connection of fabric and Velcro could be strengthened if necessary using a paperclip or hot glue.

To reduce costs, the data loggers were manually put together using components supplied by eobs-GmbH (www.e-obs.de, Gruenwald, Germany) or CellGuide Ltd. (www.cell-guide.com, Netanya, Israel). The circuit boards for GPS and acceleration measurements were obtained from e-obs GmbH and were combined with and soldered to lithium-poly-accumulators of two different capacities (260 mAh or 300 mAh at 3.7 V) and cased in heat shrink tubes of 46 mm width. Sealing with hot glue at the ends of the heat shrink tubes ensured waterproof packaging. Covering the terminal poles used for recharging with hot glue prevented the establishment of creeping currents in the field. These custom-built loggers had a total mass of between 19.09 g and 20.36 g (e-obs GmbH) or between 11.97 g and 12.83 g (CellGuide Ltd.).

In this study we used several different models of VHF-transmitters of varying weight. Transmitters were supplied by the companies ‘Andreas Wagner’ (www.wagener-telemetrie.de, weight ~ 4g), and ‘TELEMETRIE-SERVICE DESSAU’ (www.telemetrie-service.de, Dessau, Germany, weight 4 or 11 g), and we also custom built our own devices (weight ~11 g). All transmitters sent a simple short signal (150 MHz) for up to several months depending on the battery size. With the Wagener and 11 g Telemetrie-Service Dessau models it was possible to insert the wire in a tubing at the base of the VHF transmitter (Figure 3.1 A). Our custom-made devices had wires attached that were twisted with the wires on the back plate and the 4 g transmitters of Telemetrie-Service Dessau were glued directly between the spines.

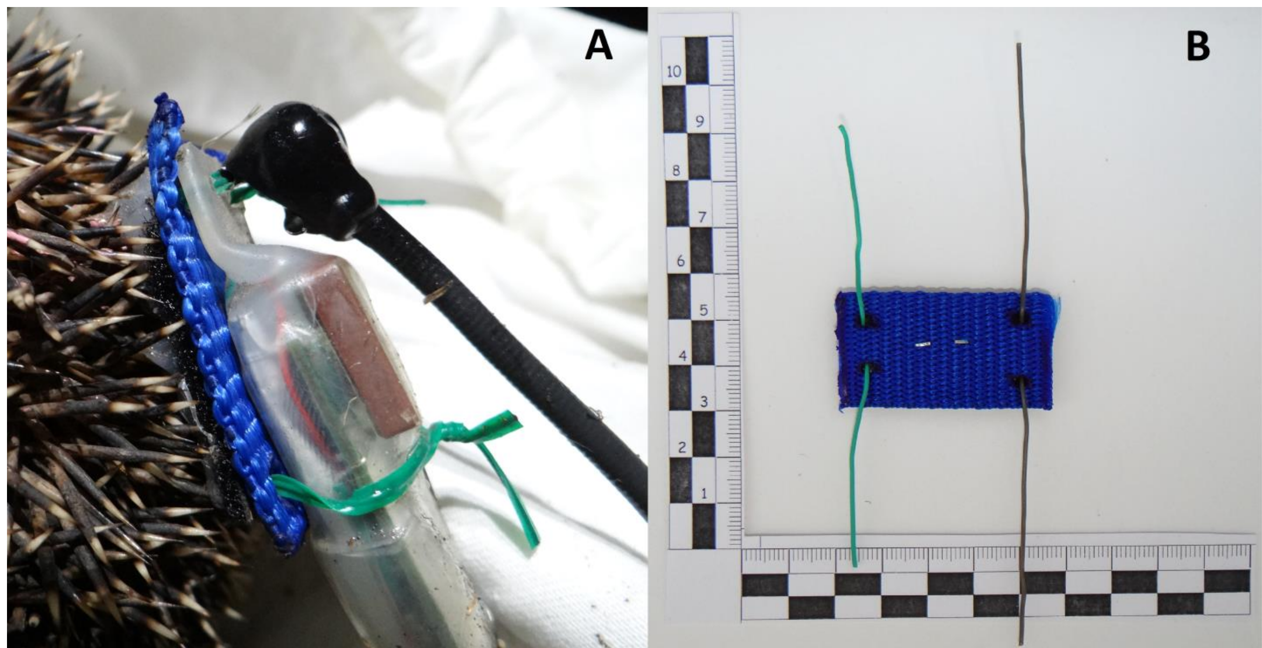


Figure 3.1: (A) A complete backpack glued to the hedgehog's spines including the back plate consisting of firmly glued fabric (blue) and loop strap (black below the blue fabric), the wires (green), the GPS device (transparent shrink tube) and the VHF transmitter (black). (B) The back plate system from above; scale numbers indicate cm. Photograph: Leon M.F. Barthel

3.3.3 **Fitting and removing of the backpack**

After capture during night surveys, and before attaching the backpack, the hedgehogs were sexed and weighed (whilst held inside a cloth bag) using a hanging scale (HDB 5K5N, Kern & Sohn GmbH, Balingen, Germany, weighing accuracy 5 g). The base plate was only attached to healthy hedgehogs with a minimum mass of 600 g. Approximately 3mm was cut from the tips of the spines using scissors to provide a larger contact area for attachment. This procedure is harmless because spines are made of keratin throughout and do not contain nerves or blood vessels. In contrast to previous studies of hedgehogs (Reeve, 1997; Warwick et al., 2006; Braaker et al., 2014; Abu Baker et al., 2016, 2017; Pettett, Johnson, et al., 2017a; Pettett, Moorhouse, Johnson, & Macdonald, 2017b), we used hot glue to attach the back plate because a mobile hot glue gun (neo1, Steinel Vertrieb GmbH, Herzebrock-Clarholz, Germany) can be used very quickly and precisely and is cost effective. Hot glue sets within seconds and is therefore much faster and deliquesces much less than other commonly used glues and epoxies; which were also tested in this project. The hot glue was applied across the complete underside of the back plate, with a thickness of about 3 to 4 mm and then pressed into the spines, ensuring that the glue surrounded all spine tips. Sometimes it was necessary to add glue from the side as well. During this procedure, we ensured that the hot glue did not come into contact with the hedgehogs' skin.

The back plate was placed on the hedgehog body at the same location as described by Recio et al., (2011), around two-thirds along the centre of the main body on its back distal to the head.

Data loggers were attached to the back plate by inserting the wires in holes on the device. The longer wires were screwed tightly around the device to fix it to the back plate and prevent loosening and wobbling. On one of the wires a VHF transmitter (with holes) could be attached to locate the hedgehogs in the field or directly clued between the spines if the model had no tubing to insert the wire. After twisting we trimmed the wires and aligned the wires with the logger. Thus, the combination of short wires aligned to the logger, a spine length away from the body ensured that the hedgehogs were not poked. After attaching the backpack, animals were re-weighed. Initial handling took a maximum of 10 min, including sexing and weighing the animal, cutting the spines and glueing the back plate onto the back of the hedgehog.

After the devices were attached, we located individual hedgehogs by their individual logger frequency with the help of a receiver (TRX-1000S, Wildlife Materials Inc., Murphysboro, IL, USA, or Wide Range Receiver AR 8200, AOR Ltd., Tokyo, Japan). Hedgehogs were tracked, recaptured and checked every day to detect whether they behaved normally or had problems to build their nests or overcome obstacles. Additionally, once a week, all hedgehogs were weighed and inspected for any problems. At the very end of the experiment, the back plate was cut off the spines below the hardened hot glue, to leave as much a length of spines as possible to ensure that the skin was not bare. In order to continue monitoring of individuals until the beginning of hibernation, we then glued another small VHF unit (~ 4 g) onto the spines using again hot glue.

3.3.4 Statistical analyses

Eighteen hedgehogs (8 females, 10 males) were initially fitted with devices for a total time of deployment of between 9 days and 42 days (Table 3.1). One hedgehog (ID 15) was not included in the data analysis because it was found dead just two days after transmitter attachment after it was run over by a train. Thus results are presented for 17 hedgehogs. To compare differences in hedgehog body masses recorded prior to the attachment and after the removal of the back plate, a Wilcoxon signed-ranks test with continuity correction was conducted. The test was performed in R version 3.4.2 using the core package (R Core Team, 2016). Results are reported as means ± standard deviation (SD).

3.4 Results

The complete backpack system once attached to the animals weighed between 25 g and 31 g; this depended on the device and the amount of glue used. The body mass of hedgehogs varied between 725 g and 1480 g (mean 972.1 ± 184.7 g, n = 17, Table 3.1), resulting in a relative mass of the complete backpack below 4.2 % of body mass. Hedgehogs with attached devices slipped under fences and crossed dense vegetation (e.g. *Hedera helix*, *Humulus lupulus*) and regularly built

new nests without showing any negative effects from the backpack system. During early trials, single spines were pulled out of the skin by the load of the backpack. This problem disappeared after applying more glue to surrounding spines near the plate.

Table 3.1: Characteristics of all study animals: ID, sex, body mass at start and end of the experiment, body mass change number of deployments, duration of complete deployment, and, for illustrative purposes, the relative mass for a 30 g back plate device combination.

Animal ID	Sex	Body mass [g]	Body mass [g]	Body mass, change [g]	Number of deployments	Duration of deployment [days]	Relative mass of the heaviest backpack [%]	
		start	end				start	end
2	f	1090	935	-155	4	41	2.7	3.2
7	f	1085	1005	-80	3	41	2.7	2.9
8	f	795	835	40	4	41	3.7	3.5
9	f	830	1010	180	4	41	3.6	2.9
13	f	725	885	160	4	41	4.1	3.3
16	f	890	1030	140	1	40	3.3	2.9
17	f	1480	1015	-465	4	41	2.0	2.9
20	f	1100	990	-110	1	40	2.7	3.0
1	m	1060	1095	35	4	41	2.8	2.7
5	m	840	850	10	1	20	3.5	3.5
10	m	1180	865	-315	0	9	2.5	3.4
11	m	900	1005	105	2	36	3.3	2.9
14	m	770	980	210	1	41	3.9	3.0
15	m	990	dead	na	0	2	3.0	na
18	m	935	1145	210	1	41	3.2	2.6
19	m	890	990	100	4	41	3.3	3.0
21	m	1015	1340	325	4	41	2.9	2.2
22	m	940	1090	150	0	28	3.1	2.7

During the study, the backpack or parts of it detached themselves on three occasions. From one hedgehog (ID 5), the backpack system had to be removed after 20 days due to dirt and the presence of fly larva under the plate. Later on, this hedgehog was recaptured twice identified by the yellow ID tubes and we observed that it had recovered completely and gained weight within two weeks. Because the field experiment had been completed, no reattachment was considered. One device was found with markings similar to that from canine teeth, indicating that maybe a predator had caught the hedgehog. Yet this individual was able to escape and was later re-caught by us and the device was reattached. In the third case, a device was found in an open meadow, including the spines to which it was glued; there was no visible reason for this detachment. The hedgehog was found later that day alive and well and the device was reattached.

Attachment and removal of GPS devices worked well as the process was swift and easy. Four backpacks had to be repaired while attached to the hedgehog, which took about 5 to 10 min of wire replacement and application of additional glue. After removing the back plate, the body

mass of hedgehogs ranged from 835 g to 1340 g (mean 1004 ± 122.7 g n = 17, Figure 3.2). Another hedgehog (ID 10) was found freshly dead, so we took the mass and used it in the analyses. There was no difference in body mass between the start and the end of device deployment of the hedgehogs (Wilcoxon signed-ranks test with continuity correction, V = 100, p = 0.28). Twelve out of 17 animals gained weight during the device deployment period. One male (ID 10) and four females (ID 2, 7, 17, 20) lost weight. This male (ID 10) was found dead on the ninth day of the experiment; the necropsy confirmed that this individual was infected by lungworms which might have already had an impact on its health and behaviour before the device had been attached. The area below the backpack of this animal showed no signs of infection.

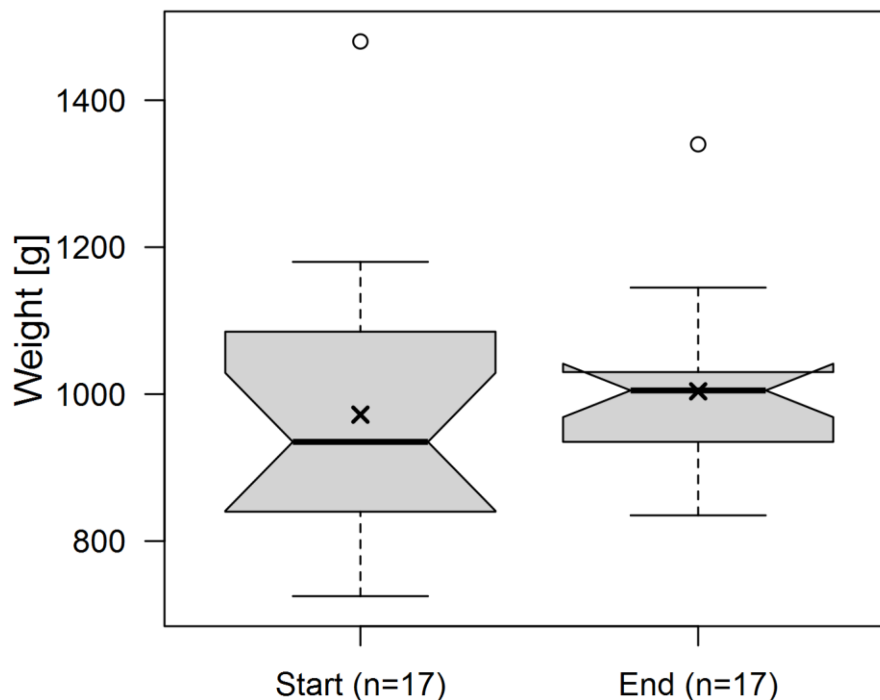


Figure 3.2: Boxplots of body mass of hedgehogs on the first day of deployment (Start) and on the last day of deployment (End). Central line: median, x: location of mean, whiskers: 1.5 times the interquartile range, circle: values more extreme than 1.5 time's interquartile range around the median.

During the study, four females (ID 2, 7, 17, 20) gave birth to hoglets, in three cases confirmed by sightings near the nest (ID 2, 7, 20) and/or by the increase in the size of teats of females (ID 2, 7, 17, 20). One female (ID 17) showed unusual behaviour in terms of restlessly moving during the whole night and during some days and died a few days before the study ended.

3.5 Discussion

European hedgehogs are an excellent example of an elusive species where data on behaviour, movement and ecology is essential for appropriate conservation management. While the UK,

Sweden and Denmark report alarming decreases in hedgehog populations, other countries cannot provide population sizes or trends because the effort required to adequately monitor hedgehogs cannot currently be undertaken (Huijser & Bergers, 2000; Hof & Bright, 2009; Hof, 2009; Johnson et al., 2015; Krange, 2015). To date, data collection on free-ranging individuals has been limited to VHF tracking or short term GPS studies, primarily due to issues of device design and attachment. Our design provides a novel way of tackling these problems using cheap and effective materials to enable long-term monitoring.

Here we used fabric material for the ground plate which was cheap, is widely available and sufficiently robust for long-term outdoor use. It is elastic, durable, breathable and easy to work with. If necessary, the colour could be suitably chosen to avoid making the animal conspicuous and more interesting for potential predators (the oddity effect e.g.; Beauchamp, 2014). Insulated copper wire of 1 mm diameter proved to be most suitable as it was the most flexible, lightweight wire that was also durable; facilitating repeated attachments.

Previous studies have commonly used fast curing epoxy for the attachment of devices to hedgehogs (Esser, 1984; Bontadina, 1991; Reeve, 1997; Warwick et al., 2006; Braaker, 2012; Braaker et al., 2012; Braaker et al., 2014; Abu Baker et al., 2016, 2017; Pettett, et al., 2017a; Pettett, et al., 2017b). However, the hot glue we used was more suitable to fix the back pack on the hedgehog's spines as it was easy, cheap and fast curing. We have had no problem in applying the glue using a small mobile glue gun, and in no case did the glue reach the skin and thus did not risk injury of the animals. Previous extensive tests of different glues and resins (Esser, 1984; Zingg, 1994) already demonstrated that epoxies suffer from long curing times, emit aerosols, generate high temperatures and require additional material to protect the animal. The only disadvantage of hot glue may be that it may not work properly if used in very wet weather conditions. We do not have enough experience with the dental composite used by Reading et al. (2016) to compare its characteristics and handling with hot glue. At 225 US$ for the initial application to 10 hedgehogs and 140 US$ for refills, dental composite is much more costly than hot glue (~ 55 US$ for 17 hedgehogs for the initial application, 0 US$ for exchanging devices on the back plate).

Our study resulted in a substantially longer duration of logger deployment than other GPS studies on hedgehogs at 42 days compared with the previous 8 days (Recio et al., 2011; Glasby & Yarnell, 2013; Braaker et al., 2014; Abu Baker et al., 2017). From our personal knowledge of many other attachment systems, the system we describe here is smaller and also enables a quick and easy exchange of data loggers, from small sensors for light, temperature, acceleration or

noise to relatively heavy GPS-loggers. The major improvement is the higher flexibility when attaching and removing devices. Other studies of hedgehogs did not reattach GPS devices to animals (Abu Baker et al., 2017) or they focused on the replacement of batteries (Boitani & Reggiani, 1984). For example, Braaker et al., (2014) reported that they reattached their devices but did not provide any details on the method. This may therefore be the first time that a fast and easy replacement of GPS data loggers on a fixed back plate has become possible, thereby enabling long-term and high-resolution studies of hedgehogs. In our study, we were able to detach and reattach rechargeable devices with short battery lifetimes in order to extend data collection. Moreover, our system provided the option of flexible solutions for potentially sensitive periods such as the mating season or lactation period, during which the behaviour, reproductive success or health of the animal might be negatively influenced by cumbersome devices. For these periods, such devices could be replaced by small and light VHF transmitters which provide the opportunity to continue monitoring the animal. Furthermore, our system permits a fast response to unforeseen situations.

Why do hedgehogs lose attachments? We suspect that the constant drag on the spines could lose either the attachment or the spine. The skin may release a single spine at any time. This may increase bending forces applied by body movements, accelerating the subsequent loosening of spines or the attachment. Such bending moments could be particularly strong that when animals curl up as then bending forces would be at a maximum.

Our mode of attachment permits short handling times and removes the need for anaesthesia. With a little bit of experience, the complete time for the initial deployment is less than 10 min. The checking and exchange of loggers on a deployed back plate took less than 1 min, including the measurement of body mass. This is amongst the fastest handling times which we are aware of and minimizing this time is desirable to reduce stress on the animal.

For hedgehogs, as small hibernating insectivores, body mass is an essential feature for assessing individual survival and fitness. Yet fluctuations in body mass can be swift and may even simply result from variable foraging success. For example, hedgehogs can increase their mass following feeding by 20 g in as little as two hours (Rautio, Valtonen, & Kunnasranta, 2013). While mass gains of up to 157 g have been reported within one night through multiple feeding events (Morris, 1985). We considered the change in body mass during our study period as a possible biomarker to assess to what extend the animals reacted to the backpack – unusually large loss of body mass could be an indicator of stress or disturbance of natural behaviour (Boitani & Reggiani, 1984; Kristiansson, 1984; Recio et al., 2011). Our modified back plate had a mass of ~2

g. Thus, our method permitted the attachment of devices with a mass of a maximum of ~28 g in order to stay below the recommended limit of 5% body mass (Sikes et al., 2011), since we stipulated that hedgehogs could only be tagged if their body mass exceeded 600 g.

In our study, we observed weight losses by 5 of 17 individuals (one male and four females), with an average mass loss of 225 g (range 80 – 465 g). For the male hedgehog (ID 10), an infection with lungworm may have exacerbated the challenges posed by the mating season and therefore instigated substantial weight loss resulting in his eventual death. Alternatively, long-term stress may have exacerbated the infection with lungworm, compromising immunocompetence (cf. a similar argument for lactating females and gastrointestinal hookworm burdens in East et al., 2015). Weight loss for all four females was most likely associated with giving birth and maternal care of hoglets as three of the four females were found with litters during the study. With animal ID 17 there were no confirmed hoglet sightings although she showed teats increased in size. The mass of hoglets at birth varies between 8 g and 25 g (Herter, 1965; Burton, 1969; Versluys, 1975; Morris, 1977) and, with an average of four hoglets per litter, there is a prospective mean weight loss of 24 g to 100 g per female. In addition, the energetically costly period of lactation results in rapid mass fluctuations for female hedgehogs (Kristiansson, 1984; Rautio, Valtonen, & Kunnasranta, 2013). Considering that these individuals were able to give birth and continued to care for their litters until the hoglets successfully left the nest, suggests that the life-history of these individuals was not substantially affected by our devices.

The placement of the system on the animal's back enabled hedgehogs to move unhindered, as they were found to undergo normal behaviour of crawling under fences and through dense vegetation. While dirt accumulated under the backpack, with regular checks of study animals any negative consequences could be prevented. This will also help to identify any possible physical deterioration from stressful responses (which we did not observe in our study) to the repeated deployment and attachment of recording devices. Since our study took place after the mating season, we do not know whether the backpack impedes mating behaviour and copulation, so this still needs to be clarified. Overall, our results demonstrate that the backpack had little influence on study animal behaviour. However, we still suggest regular re-capture of individuals to mitigate any potential negative consequences to welfare. In conclusion, we present an improved method for the attachment and reattachment of bio-logging technology to small mammals with a unique body structure.

3.6 Acknowledgements

We would like to thank Feona Oltmann, Doreen Müller, Annika Krüger, Judith Niedersen, Juline Cibis and Catrin Zordick for assistance during field work, and Indra Möllenkotte, Caroline Scholz, Cecilia Kruszynski de Assis and Shannon E. Currie for suggestions to improve the manuscript. This work was partly funded by the German Federal Ministry of Education and Research BMBF within the Collaborative Project 'Bridging in Biodiversity Science - BIBS' (grant number 01LC1501A-H) and the Leibniz Institute for Zoo and Wildlife Research (IZW). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (IZW permit 2016-02-01) and German federal law (permission number Reg0115/15 and G0104/14).

3.7 References

Abu Baker, M. A., Reeve, N. J., Conkey, A. A. T., Macdonald, D. W., & Yamaguchi, N. (2017). Hedgehogs on the move: Testing the effects of land use change on home range size and movement patterns of free-ranging Ethiopian hedgehogs. *PLoS ONE*, *12*(7), 1–18. doi:10.1371/journal.pone.0180826

Abu Baker, M. A., Reeve, N. J., Mohedano, I., Conkey, A. A. T., Macdonald, D. W., & Yamaguchi, N. (2016). Caught basking in the winter sun: Preliminary data on winter thermoregulation in the Ethiopian hedgehog, Paraechinus aethiopicus, in Qatar. *Journal of Arid Environments*, *125*, 52–55. doi:10.1016/j.jaridenv.2015.10.002

Afonso, P., Fontes, J., Holland, K. N. K., & Santos, R. S. R. R. S. (2008). Social status determines behaviour and habitat usage in a temperate parrotfish: Implications for marine reserve design. *Marine Ecology Progress Series*, *359*, 215–227. doi:10.3354/meps07272

Barron, D. G., Brawn, J. D., & Weatherhead, P. J. (2010). Meta-analysis of transmitter effects on avian behaviour and ecology. *Methods in Ecology and Evolution*, *1*(2), 180–187. doi:10.1111/j.2041-210X.2010.00013.x

Beauchamp, G. (2014). Social Predation, How group living benefits predators and prey. London, (UK): Academic Press.

Boitani, L., & Reggiani, G. (1984). Movements and activity patterns of Hedgehogs (*Erinaceus europaeus*) in Mediterranean coastal habitats. *Zeitschrift Säugetierkunde*, *49*, 193–206.

Bontadina, F. (1991). Strassenüberquerungen von Igeln Zürich. Instute of Zoology. University of Zurich.

Braaker, S. (2012). *Habitat connectivity in an urban ecosystem*. ETH Zurich. doi:/10.3929/ethz-a-009787228

Braaker, S., Kormann, U., Bontadina, F., & Obrist, M. K. K. (2017). Prediction of genetic connectivity in urban ecosystems by combining detailed movement data, genetic data and multi-path modelling. *Landscape and Urban Planning*, *160*, 107–114. doi:10.1016/j.landurbplan.2016.12.011

Braaker, S., Moretti, M., Boesch, R., Ghazoul, J., Obrist, M. K., & Bontadina, F. (2014). Assessing habitat connectivity for ground-dwelling animals in an urban environment. *Ecological Applications*, *24*(7),

1583–1595. doi:10.1890/13-1088.1

Braaker, S., Obrist, M. K., Bontadina, F., & Moretti, M. (2012). Urban connectivity. ENHANCE. Enhancing ecosystem connectivity through intervention - benefits for nature and society? Final report.

Bridge, E. S., Thorup, K., Bowlin, M. S., Chilson, P. B., Diehl, R. H., Fléron, R. W., … Wikelski, M. (2011). Technology on the move: Recent and forthcoming innovations for tracking migratory birds. *BioScience*, *61*(9), 689–698. doi:10.1525/bio.2011.61.9.7

Brooks, C., Bonyongo, C., & Harris, S. (2008). Effects of Global Positioning System Collar Weight on Zebra Behavior and Location Error. *Journal of Wildlife Management*, *72*(2), 527–534. doi:10.2193/2007-061

Burton, M. (1969). *The hedgehog*. London: Deutsch.

Cagnacci, F., Boitani, L., Powell, R. a, & Boyce, M. S. (2010). Animal ecology meets GPS-based radiotelemetry: a perfect storm of opportunities and challenges. *Philosophical Transactions of the Royal Society of London. Series B*, *365*(1550), 2157--2162. doi:10.1098/rstb.2010.0107

Collins, G. H., Petersen, S. L., Carr, C. A., & Pielstick, L. (2014). Testing VHF/GPS collar design and safety in the study of free-roaming horses. *PLoS ONE*, *9*(9), 8. doi:10.1371/journal.pone.0103189

Crofoot, M. C., Lambert, T. D., Kays, R., & Wikelski, M. C. (2010). Does watching a monkey change its behaviour? Quantifying observer effects in habituated wild primates using automated radiotelemetry. *Animal Behaviour*, *80*(3), 475–480. doi:10.1016/j.anbehav.2010.06.006

Culik, B. M., Bannasch, R., & Wilson, R. P. (1994). External devices on penguins: how important is shape? *Marine Biology*, *118*(3), 353–357. doi:10.1007/BF00350291

Dervo, B., Skei, J. K., Berg, O. K., Kraabol, M., Arnemo, J. M., & Dolmen, D. (2010). A comparison of external and internal attachments of radio transmitters on adult crested newts *Triturus cristatus*. *Amphibia-Reptilia*, *31*(2), 229–237.

Doody, J. S., Roe, J., Mayes, P., & Ishiyama, L. (2009). Telemetry tagging methods for some freshwater reptiles. *Marine and Freshwater Research*, *60*(4), 293–298. doi.org/10.1071/MF08158

East, M. L., Schulz, E., Helms, J., Thierer, D., Cablem J., Hofer H. (2015). Does lactation lead to resource allocation trade-offs in the spotted hyaena? *Behavioral Ecology & Sociobiology, 69*, 805–814.

Esser, J. (1984). Untersuchung zur Frage der Bestandsgefärdung des Igels (*Erinaceus europaeus*) in Bayern. *Berichte Der Bayerische Akademie Für Naturschutz Und Landschaftspflege*, *8*, 22–66.

Flesch, J. S., Duncan, M. G., Pascoe, J. H., & Mulley, R. C. (2009). A simple method of attaching GPS tracking devices to free-ranging lace monitors (Varanus varius). *Herpetological Conservation and Biology*, *4*(3), 411–414.

Fraser, K. C., Davies, K. T. A., Davy, C. M., Ford, A. T., Flockhart, D. T. T., & Martins, E. G. (2018). Tracking the Conservation Promise of Movement Ecology. *Frontiers in Ecology and Evolution,* 6, 150. doi:10.3389/fevo.2018.00150

Gattermann, R., Johnston, R. E., Yigit, N., Fritzsche, P., Larimer, S., Ozkurt, S., … McPhee, M. E.

(2008). Golden hamsters are nocturnal in captivity but diurnal in nature. *Biology Letters*, *4*(3), 253–255. doi:10.1098/rsbl.2008.0066

Glasby, L., & Yarnell, R. W. (2013). Evaluation of the performance and accuracy of Global Positioning System bug transmitters deployed on a small mammal. *European Journal of Wildlife Research*, *59*(6), 915–919. doi:10.1007/s10344-013-0770-3

Graham, M. D., Douglas-Hamilton, I., Adams, W. M., & Lee, P. C. (2009). The movement of African elephants in a human-dominated land-use mosaic. *Animal Conservation*, *12*(5), 445–455. doi:10.1111/j.1469-1795.2009.00272.x

Herter, K. (1965). *Hedgehogs: a comprehensive study*. London, (UK): Phoenix House.

Hof, A. R. (2009). A study of the current status of the hedgehog (*Erinaceus europaeus*), and its decline in Great Britain since 1960. Royal Holloway, University of London.

Hof, A. R., & Bright, P. P. W. (2009). The value of green-spaces in built-up areas for - western hedgehogs. *Lutra*, *52*(2), 69–82.

Hofer, H., & East, M. L. (1998). Biological conservation and stress. *Advances in the Study of Behavior*, *21*(1), 405–525. doi:10.1016/S0065-3454(08)60370-8

Huijser, M. P., & Bergers, P. J. M. (2000). The effect of roads and traffic on hedgehog (*Erinaceus europaeus*) populations. *Biological Conservation*, *95*(1), 111–116. doi:10.1016/S0006-3207(00)00006-9

Johnson, H., Reeve, N. J., Baker, P., Yarnell, R., Allen, S., & Petrovan, S. (2015). Conservation Strategy for West-European Hedgehog (*Erinaceus europaeus*) in the United Kingdom (2015-2025). London.

Kaczensky, P., Ganbataar, O., Altansukh, N., Enkhsaikhan, N., Stauffer, C., & Walzer, C. (2011). The danger of having all your eggs in one basket-winter crash of the re-introduced przewalski's horses in the mongolian gobi. *PLoS ONE*, *6*(12), 8. doi:10.1371/journal.pone.0028057

Kays, R., Crofoot, M. C., Jetz, W., & Wikelski, M. (2015). Terrestrial animal tracking as an eye on life and planet. *Science*, *348*(6240), aaa2478. doi:10.1126/science.aaa2478

Kenward, R. (2001). *A manual for wildlife radio tagging*. London, (UK): Academic Press.

Krange, M. (2015). Change in the occurrence of the West European Hedgehog (*Erinaceus europaeus*) in western Sweden during 1950-2010. Karlstad University.

Kristiansson, H. (1984). Ecology of a hedgehog *Erinaceus europaeus* population in southern Sweden. University of Lund, Sweden.

LaPoint, S., Balkenhol, N., Hale, J., Sadler, J., & van der Ree, R. (2015). Ecological Connectivity Research in Urban Areas. *Functional Ecology*, *29*, 868–878. doi:10.1111/1365-2435.12489

Morris, P. A. (1977). Pre-weaning mortality in the hedgehog (*Erinaceus europaeus*). *Journal of Zoology*, *182*(2), 162–164. doi:10.1111/j.1469-7998.1977.tb04150.x

Morris, P. A. (1985). The effects of supplementaey feeding on movements of hedgehogs (<i>*Erinaceus europaeus*). *Mammal Review*, *15*(1), 23–32.

Pearl, M. C. (2000). Research techniques in animal ecology methods and cases in conservation science. *The Journal of Wildlife Management*, *65*(3), 599. doi:10.2307/3803113

Pettett, C. E., Johnson, P. J., Moorhouse, T. P., Hambly, C., Speakman, J. R., & Macdonald, D. W. (2017a). Daily energy expenditure in the face of predation: hedgehog energetics in rural landscapes. *The Journal of Experimental Biology*, *220*(3), 460–468. doi:10.1242/jeb.150359

Pettett, C. E., Moorhouse, T. P., Johnson, P. J., & Macdonald, D. W. (2017b). Factors affecting hedgehog (*Erinaceus europaeus*) attraction to rural villages in arable landscapes. *European Journal of Wildlife Research*, *63*(3), 12. doi:10.1007/s10344-017-1113-6

R Core Team. (2016). R: A language and environment for statistical computing.

Rautio, A. (2015). On the northern edge – ecology of urban hedgehogs in eastern Finland. University of Eastern Finland.

Rautio, A., Valtonen, A., & Kunnasranta, M. (2013). The effects of sex and season on home range in European hedgehogs at the northern edge of the species range. *Annales Zoologici Fennici*, *50*(1–2), 107–123. doi:10.5735/086.050.0110

Reading, R. P., Kenny, D., Murdoch, J., & Batdorj, S. (2016). Use of dental restorative temporization material for attaching radiotransmitters to hedgehogs. *Wildlife Society Bulletin*, *40*(2), 355–358. doi:10.1002/wsb.655

Recio, M. R., Mathieu, R., & Seddon, P. J. (2011). Design of a GPS backpack to track European hedgehogs *Erinaceus europaeus*. *European Journal of Wildlife Research*, *57*(6), 1175–1178. doi:10.1007/s10344-011-0530-1

Reeve, N. J., Bowen, C., & Gurnell, J. (2015). The Regents's Park hedgehog project; Identification marking 2015., 1–2.

Reeve, N. J. (1994). *Hedgehogs*. T & A. D Poyser.

Reeve, N. J. (1997). The Survival and Welfare of Hedgehogs (*Erinaceus europaeus*) After release back into the wild. *Animal Welfare*, *7*, 189–202.

Scheibe, K. M., & Gromann, C. (2006). Application testing of a new three-dimensional acceleration measuring system with wireless data transfer for behavior analysis. *Behavior Research Methods*, *38*(3), 427–433.

Schneirla, T. C. (1950). The relationship between observation and experimentation in the field study of behavior. *Annals of the New York Academy of Sciences*, *51*(6), 1022–1044. doi:10.1111/j.1749-6632.1950.tb27331.x

Shamoun-Baranes, J., Bom, R., van Loon, E. E., Ens, B. J., Oosterbeek, K., & Bouten, W. (2012). From sensor data to animal behaviour: An oystercatcher example. *PLoS ONE*, *7*(5), e37997. doi:10.1371/journal.pone.0037997

Shilova-Krassova, S. A. (1952). The nutrition of the hedgehog (*Erinaceus europaeus L.*) in southern forests. *Zoologicheskii Zhurnal*, *2*(31), 944–947.

Sikes, R. S., Gannon, W. L., & Animal Care and Use Committee. (2011). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy*, *92*(1), 235–253. doi:10.1644/10-MAMM-F-355.1

Vandenabeele, S. P., Shepard, E. L. C., Grémillet, D., Butler, P. J., Martin, G. R., & Wilson, R. P. (2015). Are bio-telemetric devices a drag? Effects of external tags on the diving behaviour of great cormorants. *Marine Ecology Progress Series*, *519*, 239–249. doi:10.3354/meps11058

Vandenabeele, S. P., Wilson, R. P., & Wikelski, M. (2013). New tracking philosophy for birds. *Frontiers in Ecology and the Environment*, *11*(1), 10–12. doi:10.1890/13.WB.002

Versluys, S. D. W. (1975). Wel en wee van de egel. In *Diergeneeskunding Memorandum 22nd Year* (pp. 235–301).

Voigt, C. C., Thalwitzer, S., Melzheimer, J., Blanc, A.-S., Jago, M., & Wachter, B. (2014). The Conflict between Cheetahs and Humans on Namibian Farmland Elucidated by Stable Isotope Diet Analysis. *PLoS ONE*, *9*(8), e101917. doi:10.1371/journal.pone.0101917

Warner, D. A., Thomas, J., & Shine, R. (2006). A simple and reliable method for attaching radio-transmitters to lizards. *Herpetological Conservation and Biology*, *1*(2), 129–131.

Warwick, H., Morris, P. A., & Walker, D. (2006). Survival and weight changes of hedgehogs (*Erinaceus europaeus*) translocated from the Hebrides to Mainland Scotland. *Lutra*, *49*(2), 89–102.

Wilson, D. E., Cole, F. R., Nichols, J. D., Rurdran, R., & Foster, M. S. (1996). *Measuring and monitoring biological diversity. Standard methods for mammals*. Washington: Smithsonian Institution Press.

Wilson, R. P., & McMahon, C. R. (2006). Measuring devices on wild animals: What constitutes acceptable practice? *Frontiers in Ecology and the Environment*, *4*(3), 147–154. doi:10.1017/CBO9781107415324.004

Zingg, R. (1994). Aktivitaet Habitat- und Raumnutzung von Igeln (*Erinaceus europaeus*) in einem ländlichen Siedlungsgebiet. Universität Zürich.

Figure 3.3: Hedgehog with the back plate system

4 Distinguishing spatial from temporal effects in disturbance biology: Hedgehogs in the urban matrix of habitat fragmentation and noise pollution

Draft

Barthel, Leon M.F. [1,3]; Gras, Pierre [2,3] ; Rast, Wanja [1]; Berger, Anne [1,3]; Hofer, Heribert [2,3,4,5]

[1] Department of Evolutionary Ecology, Leibniz Institute for Zoo and Wildlife Research (IZW), Alfred Kowalke Straße 17, 10315 Berlin, Germany
[2] Department of Ecological Dynamics, Leibniz Institute for Zoo and Wildlife Research, Alfred Kowalke Straße 17, 10315 Berlin, Germany
[3] Berlin Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany
[4] Department of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Takustrasse 3, 14195 Berlin, Germany
[5] Department of Veterinary Medicine, Freie Universität Berlin, Oertzenweg 19b, 14195 Berlin, Germany

4.1 Abstract

Anthropogenic activities can result in both transient and permanent changes in the environment. Whereas temporal disturbances typically create a transient habitat change, spatial disturbances such as fragmentation are often of a permanent nature or even intensify. Temporal disturbances are frequently studied with a focus on stress and welfare whereas spatial changes such as habitat fragmentation are usually investigated in the context of conservation-oriented population viability, yet wildlife populations are regularly subjected to both types of habitat change. Therefore, a unified framework to study the response of wildlife to different types of disturbance and its resulting habitat change seems desirable. We studied spatial and temporal behavioural responses in terms of foraging movements and nesting behaviour of European hedgehogs to a transient (open-air music festival) and a permanent (fragmented landscape) disturbance in the conurbation of Berlin, Germany, in two distinct habitats and during two years using a Before and After and Control and Impact study design (BACI). Confronted with the music festival, hedgehogs substantially changed their movement behaviour and nesting patterns and substantially decreased the degree of functional coupling (DFC) of their activity patterns, suggesting that this was a substantial stressor and that they had to re-evaluate the trade-off between foraging success and risk aversion. Hedgehogs in a fragmented area used larger home ranges and moved with higher speed but otherwise showed behaviours and high DFCs similar to individuals in an unfragmented environment, suggesting that fragmentation posed a moderate challenge with which they could cope. The unpredictable acute yet transient habitat change (disturbance) affected by the music festival had a more substantial effect than the static disturbance through fragmentation, to which hedgehogs had a long time to adjust to. We conclude that it is valuable to view and study disturbance from a unified perspective as a process

of habitat change, that an observed response to one form of habitat change (disturbance) may not necessarily predict the degree and intensity of response to other forms of habitat change (disturbance), that males and females may differ in their responses, and that detailed automated recordings of foraging movements using GPS-devices combined with 3D-accelerometers can reveal the degree and intensity by which wildlife responds to and copes with particular forms of disturbance. Such a perspective will benefit both studies on welfare and the conservation of threatened species.

Keywords: Disturbance, fragmentation, anthropogenic stressors, urban ecology, behavioural plasticity, GPS telemetry, hedgehogs

4.2 Introduction

Humans change their environment in different ways either slowly and indirect, e.g. through changes over the last few centuries, or fast and direct within a few days when building a road or removing a forest patch (McDonnell and Hahs, 2015; Wong and Candolin, 2015; Hastings et al., 2018). Faster changes may pose larger problems than slow changes as they require individual animals to show appropriate behavioural flexibility or plasticity within their own lifetime to respond appropriately and cope. This flexibility could contribute to better population viability in the face of frequent or regular disturbances. A disturbance describes every change in an environment that poses a change in the ecosystem (see Rykiel, 1985). In human-modified ecosystems, disturbances can range from light, noise, over air pollution to habitat fragmentation and a host of other processes (Walker, 2012; McDonnell and Hahs, 2015). When habitats are disturbed (and thus changing), animals must in principle reconsider trade-offs between foraging success and risk aversion similar to the urban landscape of fear (Bleicher, 2017; Stillfried et al., 2017). Wildlife and wildlife populations have several options to respond to various forms of disturbance: within a lifetime of an individual, disperse and seek another place to live or stay put and adjust through behavioural plasticity. In addition, via several generations, genetic changes may be possible to genetically adapt to the new environmental conditions if they are permanent (Wong and Candolin, 2015).

However, it is challenging to disentangle temporal, acute and permanent stressors and test their effects within one species. In this study, we aim to investigate the effects of habitat fragmentation and a temporal disturbance (noise and increased human presence) in European hedgehogs (*Erinaceus europaeus*) who already live in an urban conurbation. For this purpose, we monitored the behaviour of European hedgehogs in two populations in Berlin where one population faced fragmentation and the other faced several presumed stressors associated with a

music festival. In particular, we investigated the spatial, temporal and behavioural response of hedgehogs in a substantially anthropogenic modified environment, urban (woodland) parks.

Festivals often take place in open green areas that are normally open for other public purposes or, sometimes, are not accessible to the general public. Hosting a festival implies that in a specific area heavy machines will be used by many people present in order to set up stages and other focal areas of amusement within a short time frame. During a festival, a huge crowd of people wanders around the festival site, loud music plays through most of the day and night and for enjoyment and safety, the whole area is lit up and fenced. Dependent on the animal species, they are therefore confronted with additional noise, light and the presence of people.

Fragmentation is created by transforming habitats important to a particular species into smaller patches, thereby creating a mosaic-like landscape (Doncaster and Dickman 1987; Wilcove et al. 1986; Fahrig 2003). Thus, fragmentation can impede movement among resource patches and limit access to mating partners (Banks et al., 2007; Shepard et al., 2008). Animals may be substantially affected by habitat fragmentation if remaining semi-natural food patches are too far away (Baker and Harris, 2007). Some species can adjust their behaviour to these circumstances (Lowry, Lill and Wong, 2013) but it can lead to an "ecological trap" (Wong and Candolin, 2015). Some examples of behavioural plasticity include increasing home ranges and adjusting activity rhythms to cope with fragmentation (Hertel et al., 2016; Hertel, Swenson and Bischof, 2017; Soanes et al., 2018). These phenotypic changes induced by anthropogenic factors are generally greater than on natural habitat changes (Wong and Candolin, 2015).

The European hedgehog (*Erinaceus europaeus*) lives at higher abundances in villages and human settlements than rural areas (Dowding et al., 2010; Hubert et al., 2011; Pettett, Moorhouse, et al., 2017). Possible reasons are predator avoidance (Doncaster 1994; Krange 2015), as predators such as the Eurasian badger (*Meles meles*) are rarer within conurbations, and the positive effects of the mosaic-like structure offered by conurbations, where bushes and vegetation for daily nests and open or semi-open areas to search for food are readily available in many areas (Kristiansson, 1984; Hubert et al., 2011). Hedgehogs can also move out of unfavourable habitats (Doncaster, Rondinini, and Johnson 2001). These traits and the relatively small home range size make hedgehogs a good model species for urban wildlife-human interactions of small mammals. Usually, urban parks are favourable environments for hedgehogs because they provide food and resting places and have no big artificial barriers. The mosaic-like structure can also pose problems since dispersal out of unfavourable habitats can be limited if the urban matrix provides a very patchy environment with only small, isolated patches of suitable habitat and evolutionary

adaptations will require many generations (Wong and Candolin, 2015). Therefore, it is at present unclear how wildlife such as hedgehogs cope with habitat fragmentation as an example of environmental disturbance imposed by anthropogenic changes in conurbations in that good habitat such as parks are interspersed with many urbanized structures surrounding parks.

To investigate the behavioural plasticity in response to acute and permanent habitat changes, we analysed the behavioural response on different levels. First, we used GPS data to investigate the home range area and movement behaviour of hedgehogs which is closely related to foraging (Braaker et al., 2014). Second, we assessed the occurrence and circadian rhythmic pattern of specific behaviours because hedgehogs are known to be strictly nocturnal and a reduction in the occurrence of specific behaviours in challenging circumstances are considered to be a sign of stress (Reeve 1994; Morris 1997) even if hedgehogs are recognised to show behavioural plasticity (Dowding et al., 2010). Third, we monitored nesting behaviour. Day nests of hedgehogs are important because they are used by all adults as resting sites during the day, by females also as the locality where young are kept. Hedgehogs sleep in them almost every day and typically reuse the same nest for several successive days (Reeve and Morris, 1985; Haigh, O'Riordan and Butler, 2012).

The studies were performed in two different habitats to investigate two scenarios: We studied hedgehogs in a large Berlin urban park, the Treptower Park, with little habitat fragmentation and low noise levels at night during normal times where the authorities permitted the event of a huge music festival with over 140,000 visitors, and investigated the effects of habitat fragmentation in another large urban park, the Tierpark Berlin, with many daytime visitors but very low nightly disturbance from people. We hypothesised that disturbance influenced movements in terms of space use, activity patterns and nesting behaviour, behaviours which – particularly during the reproductive season – are likely to have fitness consequences (Lowry, Lill and Wong, 2013).

4.2.1 **Predictions**

In the following, we present the predictions for (A) the acute disturbance (habitat change) as presented by the music festival, and (B) the permanent (chronic) habitat change in terms of park fragmentation:

A: In the acute habitat change, hedgehogs need to re-evaluate the trade-off between foraging profitability and safety by reducing the vicinity to people or anthropogenic sources of disturbance, and reconsider options for easily accessible food.

(1) Regarding the space use we think that hedgehogs are able to move out of the

unfavourable habitat and thus could either leave the area or show adjustment of movement behaviour: (1a) We predict avoidance of the previously favoured area which would result in shifting the centre of the home range area used, detectable by shifting centroids of nightly calculated Minimum Convex Polygons. (1b) Another co-occurring or independent adjustment could be a decrease in the nightly used home range area.

(2) We propose that hedgehogs adjust their movement behaviour. The animals now have to look for the same amount of food in a potentially less favourable and/or smaller area and thus foraging effort may have to be increased. We, therefore, predict under disturbance an increase in computed search intensity, higher turning angles and slower speed.

(3) The relative time spent in various behaviours will be shifted towards higher vigilance, or general levels of activity will be reduced (Risk aversion).

(4) We predict that high levels of disturbance during the festival induce females and males to switch their nests more often and the number of days spent in the same nest decreases.

B: Habitat fragmentation (chronic habitat change).

(1) As the movement of hedgehogs is strongly associated with linear structures (Dowding et al., 2010), fragmentation will increase the area of space that is of no interest to hedgehogs and thus increase the distances they have to cover. Thus, under fragmentation, the home range area would be bigger than in the non-fragmented park. This is not the case if the hedgehogs had access to one very big food patch which we consider to be unlikely (Luniak 2004).

(2) As fragmentation is likely to increase distances for commuting between favourable food patches, movement characteristics will be affected, such as higher speed, a larger number of smaller turning angels and in general a lower search intensity than in a non-disturbed non-fragmented habitat.

(3) Behaviour, in general, should not be different but due to the used area behaviour could change accordingly.

(4) Fragmentation may influence nesting behaviour in such a way that animals either have to

change their nests more frequently to be closer to favourable food patches, or extend their stay in nests if they are close to favourable food patches.

4.3 Methods

4.3.1 Fieldwork

Fieldwork was conducted between 10 August and 20 September 2016 in the Treptower Park, in southeast Berlin, Germany (52.48846°N, 13.46974°E) and in August and September in 2017 in the Tierpark, a big park containing a zoological garden in East Berlin, Germany (52.50326°N, 13.52976°E). Both parks have variable green spaces from short meadow over variable shrubs and hedges to shrubs and include big trees. Additionally, there are playgrounds, larger sealed areas and footpaths. Treptower Park, the site where the music festival took place, is open to the general public 24 hours and 7 days per week whereas the Tierpark is closed to the general public from dusk to the morning. The Tierpark contains numerous animal enclosures, small buildings and many more concrete footpaths, creating a mosaic-like fragmented habitat with many non-accessible areas. The maintenance of the parks is similar, with leaf litter being removed from some areas, particularly the footpaths, and being left in bushes and scrub throughout the year, offering a similar habitat in both parks suitable for hedgehogs and other wildlife.

In 2016 the Lollapalooza Festival with over 140.000 visitors took place in the Treptower Park. From 29 August to 16 September, a substantial portion of the park (excluding a war memorial and the south-eastern segment) was massively changed. Music stages, amusement facilities and enclosures were constructed and built between 9 August and 09 September, the festival took place on 10 and 11 September, and deconstruction of all facilities took place from 12 September onwards. We collected data on hedgehog movements and behaviour before the festival (pre-festival) until construction work for the festival started and during the festival-phase, including the time periods of construction and deconstruction. The pre-festival period is defined from 10 August until 28 August and represents the control for both the transient disturbance caused by the festival and, as an example of hedgehogs living in an unfragmented and undisturbed urban habitat, for the hedgehogs studied in the Tierpark.

Hedgehog capture and logger attachment

At the beginning of each study period, surveys were carried out during two to three nights at least one hour after sunset to find active hedgehogs by spotlighting (P14.2, LED Lenser, Solingen, Germany). Every hedgehog located was marked with five shrink tubes on the spines (Mori *et al.*, 2015). The tubes were labelled with a number starting with 1 to identify them during

recapture (N. J. Reeve, pers. comm. 2016). From all previously captured hedgehogs, we selected eight hedgehogs (four of each sex) and equipped them with GPS/ACC loggers (E-obs GmbH, München, Germany) and VHF transmitters using a backplate system (Barthel, Hofer and Berger, 2018). We only used hedgehogs with a body mass exceeding 600g to ensure that the attached logger equipment fell below the 5 % body mass rule (Hofer and East, 1998; Sikes et al., 2011).

During the study, all hedgehogs were weighted and inspected for any problems once a week; these occasions were also used for the necessary reloading of the GPS/ACC data loggers. Nesting behaviour was recorded every day by locating the VHF signals of each hedgehog carrying a logger (TRX-1000S, Wildlife Materials Inc., Murphysboro, IL, USA, or Wide Range Receiver AR 8200, AOR Ltd., Tokyo, Japan).

Logger/ Sampling Setup setup / ACC

GPS positions were taken during expected activity times of hedgehogs from 1900 hours to 0700 hours in 10 min intervals, in bursts of five points. VHF transmitters continuously broadcast signals throughout the whole study period. Acceleration data was recorded alongside GPS data every minute. These accelerometers are programmed to record a short burst of high-resolution data. A sampling frequency of 100 Hz per axis was chosen for the present study. All three available axes were measured simultaneously. A burst took 2.64 s for two individuals, the other individuals were recorded with bursts of 2.5 s. This difference in burst length is not ideal, although the burst length is only important for three out of 25 predictors used for the model (see chapter "Behaviour prediction and budget"). Bursts were recorded every minute.

To account for missing data because of recharging or logger malfunctioning, all data with less than 1430 (1440 for complete 24 h) measurements between 00:00 and 23:59 were removed from the data set. This removal of data ensured that only days with a comparable length and with the same number of records during days and nights were considered for the analysis and therefore did not favour behaviours that only occurred during a specific time of the day.

Nesting Behaviour

Nests of hedgehogs were recorded every day. The position was recorded using a *Garmin GPSmap 60CSx* device by Garmin Deutschland GmbH, Germany. For this study, relevant data were recorded alongside some other traits (Supplementary Table 4.11) and were checked every day for the nest the hedgehogs have slept in. If a hedgehog was found in the vicinity (2 m) of a nest without a new nest, the existing nest was noted as the day nest of the hedgehog. Some animals had to be removed from the dataset of 2017 in the fragmented habitat because they occupied

fewer than 5 nests and lost their VHF transmitter. If a hedgehog lost a transmitter and could not be found at night to re-attach the transmitter, or night work had been discontinued, the nest surveys for the individual were stopped.

4.3.2 Analyses

GPS Data

For all comparison within the acute habitat change, all data of the particular park were used; in comparisons, with the fragmented park, only the data from the pre-festival period was used. Mean points of the GPS data collected within 10 s were calculated. To conserve the natural variability we decided to use every night as a single event and calculated the following values accordingly using R (R Core Team, 2018). To assess the nightly used area we calculated the 95 % Minimum Convex Polygon (MCP95) and the Kernel density estimates (kde50) as a core area of use (Calenge, 2006; R Core Team, 2018). In both cases, we used a linear model (Pinheiro et al., 2018) to perform a linear mixed effects analysis on the relationship between used area (mcp95 or kde50) and our three treatments. As fixed effects, we entered treatment and sex with interaction into the model. As random effects, we had intercepts for individuals. To fit the assumptions we included a power function using varPower() as weights. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained calculating an analysis of variance (Fox and Weisberg, 2011) followed by a general linear hypothesis and multiple comparisons (Hothorn, Bretz and Westfall, 2008) using a matrix with only relevant comparisons between following groups: pre vs. pre comparing both sexes, pre vs. festival within sex, pre vs. fragmented within sex, fragmented vs. fragmented comparing both sexes (Figure 4.1).

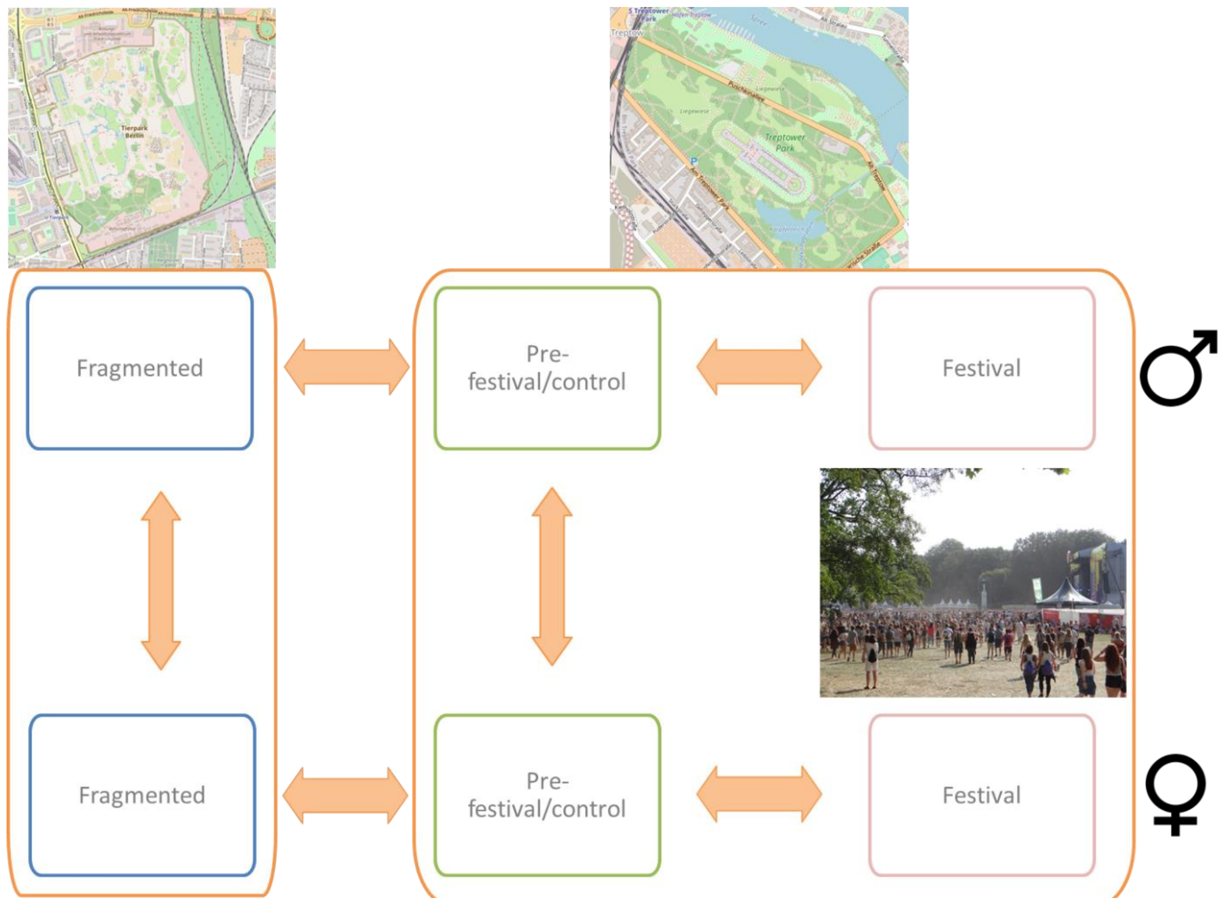


Figure 4.1: statistical study design, in green the control from the pre-festival period in the Treptower Park is compared to the festival dataset (reddish) and the fragmented dataset of the Tierpark (blue), arrows indicate statistical comparisons.

We proceeded in a similar manner when analysing the movement speed of hedgehogs as a travelled distance for a time interval between GPS positions [m/s]). We used the lme4 package to perform a linear mixed effects analysis on the relationship between speed and treatment (Bates et al., 2015). As fixed effects, we entered treatment and sex (with interaction) and as random effects, we had intercepts for individuals. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. P-values were obtained by applying an analysis of variance and running general linear hypotheses and multiple comparisons (Hothorn, Bretz and Westfall, 2008) using similar comparisons as above.

To evaluate how animals use the available habitat we calculated a ratio of area used (mcp95 in [m^2]) and distance travel [m] per night (calculated with st_length, Pebesma 2018), resulting in a measure of search intensity with units [$m/(m^2*d)$] or moved distance per square meter and day. To evaluate whether treatment or sex had an effect on this parameter we used the SpaMM package (Rousset and Ferdy, 2014) by first finding the right fit and then comparing the null model with different models.

To detect wherever the hedgehogs shifted their utilized area, the longitudinal and latitude values of centroids per night were used separately using function centroid() (Hijmans, 2017). We normalized the values by subtracting the mean value from the pre-festival phase and worked with the absolute values. For the latitude and longitude values, linear mixed effect models were fitted. Both time values hat to be square root transformed before fitting the model to meet the assumptions of homoscedasticity or normality. P-values were obtained from an analysis of variance (Fox and Weisberg, 2011).

Movement of hedgehogs was further characterized by calculating turning angles (Michelot, Langrock and Patterson, 2016) and plotted as absolute values because we were interested in the general movement. Results were then randomly sampled and compared in a permutation approach 1000 times using a two-sample two-sided Kolmogorov-Smirnov test (R Core Team, 2018). The comparison was only done on the treatment level (pre-festival vs. festival and pre-festival vs. fragmentation).

ACC/acceleration data analyses

Behaviour prediction and budget

We used a supervised machine learning algorithm for behaviour detection. The train and test dataset for the behaviour recognition were taken from a previous study. The whole procedure is described in Chapter 5. By joining multiple Support Vector Machines (SVM) the selected behaviours were classified. Here, we considered three behaviour classes: resting, balling up and locomotion (referred to as walking). To account for behaviours that are not included in the model but might occur in hedgehogs, a threshold for the probability belonging to a class of 0.7 was set for the SVM. Otherwise, the behaviour was classified as "other" behaviour.

In addition, the raw data were tested for missing measurements within the bursts. All bursts where fewer data were recorded than intended by the settings were removed (264). The SVM model was then used to assign a behaviour prediction to every burst and its corresponding time stamp. The behaviour of every individual was treated for the following tests separately. To test for effects on behaviour classes, a general linear model was performed (Bates et al., 2015) taking a quotient of the behaviour in relation to all behaviours. As a fixed effect, the treatment, as well as the sex and the interaction, were put into the model. Individuals were included as random effect. This was followed by an analysis of variance (Fox and Weisberg, 2011) and general linear hypotheses and multiple comparisons (Hothorn, Bretz and Westfall, 2008) using the same matrix as before.

Stress detection via the Degree of Functional Coupling (DFC)

The Degree of Functional Coupling (DFC) is a measure for the synchrony of (internal) cyclic behaviour and the (external) environmental 24 h period (Sinz and Scheibe, 1976; Berger et al., 1999; Scheibe et al., 1999). To calculate DFCs, the standard deviation of raw acceleration data of all three axes were calculated and summed up per measurement interval. Following the protocol of Berger et al. (Berger et al., 2003; Berger, 2011), this time series was autocorrelated in order to filter out the noise and enhance rhythmic components. Afterwards, a Fourier transformation was used to break it down into its rhythmic components, as described by the percentage of each component in the original time series. The longest Fourier period tested covered the entire length of the autocorrelation function (here three days); the shortest Fourier period tested was twice the sampling interval (here 2 minutes). The DFC is then calculated by dividing the portion of Fourier transformation components that harmonize with the 24-hour rhythm by the entirety of the Fourier spectrum. To gain an adequate statistical power of the 24 hour period, DFC were calculated for time series of three days equivalent to the procedure of a moving average (first data set covers day 1 to 3, second data set covers day 2 to 4 and so on). The resulting DFCs were assigned to the day of the three days that entered the calculation for the first time. These data were then analysed using a linear mixed effect model with treatment and sex and their interaction, the values had to be Arcsine transformed in order to meet the assumptions of homoscedasticity and normality. Afterwards, an analysis of variance (Fox and Weisberg, 2011) and general linear hypotheses and multiple comparisons (Hothorn, Bretz and Westfall, 2008) was performed.

Nesting behaviour

For each nest, the duration of occupation was scored as exact if both starting and stopping dates of nest use were recognised, or as right-censored (a minimum estimate), if either the starting date or the stopping date at the beginning and end of study periods were not known. Then, the survivorship function was calculated using package survival (Therneau, 2015) separately for both parks and treatment conditions. If significance was found a post hoc Mantel test was performed to detect the source of the difference.

4.4 Results

4.4.1 Movement data

Data of 16 hedgehogs with between 3 and 41 nights per animal were collected resulting in 426 tracked nights in total including 156 nights for the control, 152 for the festival and 118 for the fragmented studies. Sexes are represented with 236 females and 190 male data points.

The roamed area (Figure 4.2 A) measured by the mcp95 was significantly affected by treatment ($\chi2 = 54.82$, df = 2, $p < 0.001$) and sex ($\chi2 = 6.48$, df = 1, P = 0.011). Treatment was similar between sexes ($\chi2 = 1.7432$, df = 2, P = 0.42). A post-hoc test revealed females in the control group used smaller areas by 1.9 times than males in the control group (2.55 ha to 4.71 ha, respectively). While both sexes decreased their area during the festival (acute change (Estimate (E) = -1.0290, std. Error (se) = 0.2214, z value (z)= -4.648, Pr[>|z|] <0.001, and males -1.4665 0.3770 -3.890 <0.001) only females increased the area in the fragmented habitat (1.9421 0.6945 2.796 0.0283). Male hedgehogs already occupying a bigger area than females in the control and showed only a slight increase to the control group (0.7302 0.8663 0.843 0.9200). While males and females in the fragmented habitats had similarly sized home ranges (0.6849 0.8706 0.787 0.9390). These were replicated by the used core area measured by the kde50, except for the comparison between pre-festival and fragmented within the females (Supplementary Figure 4.2).

Looking into the general habitat effects on movement behaviour (Figure 4.2 B – D), speed was affected by treatment ($\chi2 = 33.3$, df = 2, $p < 0.001$) but not by sex ($\chi2 = 1.06$, df = 1, p = 0.303) or their interaction ($\chi2 = 1.234$, df = 1, p = 0.5396). During the festival, hedgehogs had lower speeds (0.038 m/s ± 0.008) and hedgehogs in the fragmented habitat (0.0485 m/s ± 0.001) were, in general, moving faster than in the control group (0.0404 m/s ± 0.008) (Figure 4.2 B). Search intensity [m/m^2*d] (Figure 4.2 C) was also only affected by treatment ($\chi2 = 7.4195$ df= 2 p= 0.02448) and neither by sex nor by the interaction ($\chi2 = 0.1074$ df= 1 p= 0.74315, $\chi2 = 3.2151$ df= 2 p= 0.20037 respectively). The mean value was the lowest for the control and the highest for the festival. The largest confidence interval has a fragmented value. All these movement difference should add up into the characteristics of the movement, for example, the turning angles of hedgehogs (Figure 4.2 D). Even if the curves of the subsample look slightly different there was no difference in the characteristics of the turning angles (Figure 4.2 D).

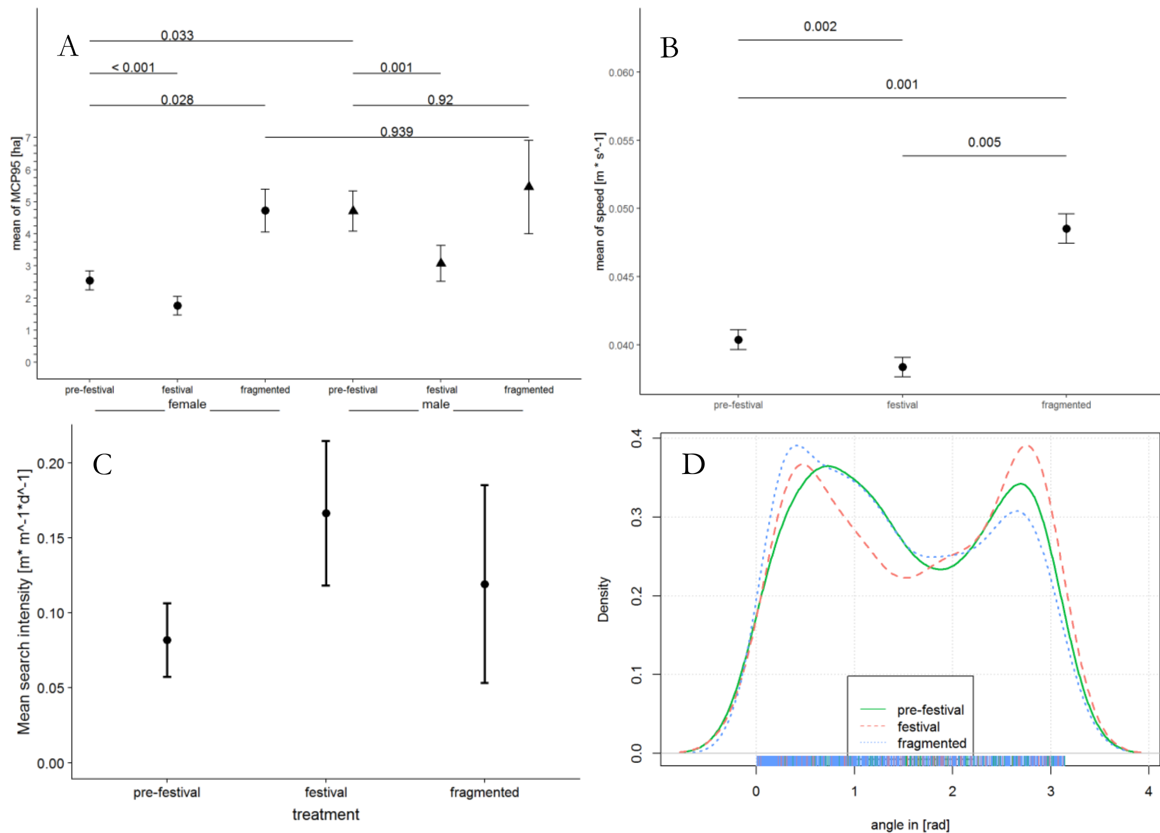


Figure 4.2: Results of the GPS data A: MCP 95 indicating Measurements for used area dots representing females, triangles males; B: mean of speed [m/s] over both sexes against treatment C: mean of search intensity [m/(m²*d)] over both sexes against treatment; dots/triangles indicate mean values; whiskers are confidence interval, D: Distribution of absolute turning angles showing one of the subsamples that were tested

A shift in the utilized area

The centroid values of the daily used area present the mean point of the used area and should have shifted if the hedgehogs used other areas, and the distribution should change if they avoid certain areas. In the control, hedgehogs facilitated a central big open meadow and both sexes had a near to normal distribution in their longitudinal values (Figure 4.3). While facing the acute changes of the festival hedgehogs went further away from their overall mean centroid by on average ~35/30 m (longitudinal/latitudinal) in female and in males more than 65/105 m (effect of treatment latitude χ^2= 80.5897, (1) p < 2.2e-16/ longitude χ^2= 80.858, (1) p < 2.2e-16, and the interaction of treatment and sex (χ^2=21.4375, (1) p = 3.655e-06 / χ^2=11.790, (1) p = 0.0005954).).

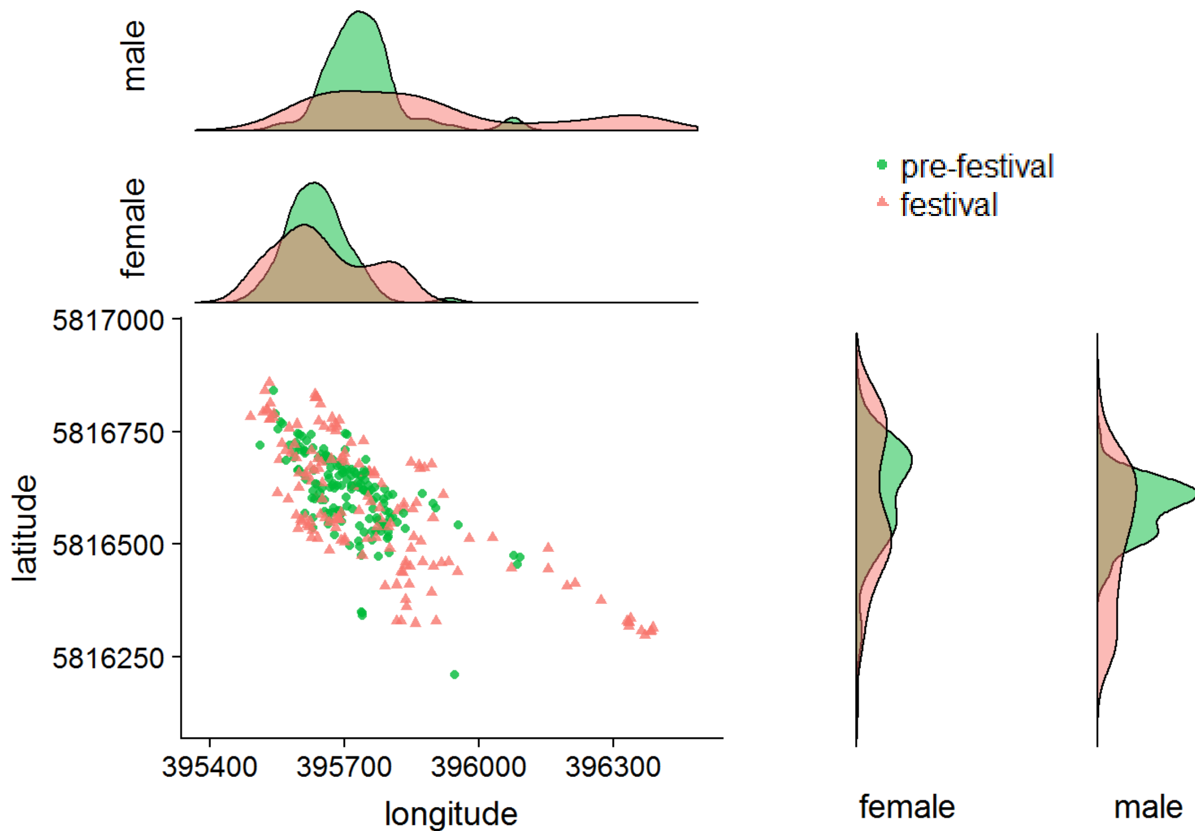


Figure 4.3: Centroid distribution circle/reddish = pre.-festival, triangle/bluish = festival, density shows kernel density estimate, all y-axes in the density plots show the same range.

Behaviour

In all considered behaviours (balled up, walking, resting, other - Supplementary) we only found an effect in treatment and in the case of balling up and resting in the interaction (Figure 4.4). Differences occurred between pre-festival and festival. In females and males balling behaviour was detected more frequently during the festival than in the control phase for either sex, females showed an increase of 0.153 and males 0.2. With regard to the walking behaviour, only males showed an increase of 0.03. Resting behaviour was identified less in both sexes during the festival with 0.21 in females and 0.014 in males. All these behaviours are part of the daily rhythm of the hedgehogs which is also represented in the DFC value. The only recorded effect was seen due to treatment, where both sexes showed the same patterns. Both sexes showed similar values in the control and the fragmented environment, while the values during the acute event were lower than the control. The highest values where observed in the fragmented habitat, while males in the fragmented habitat had lower values with a higher variation.

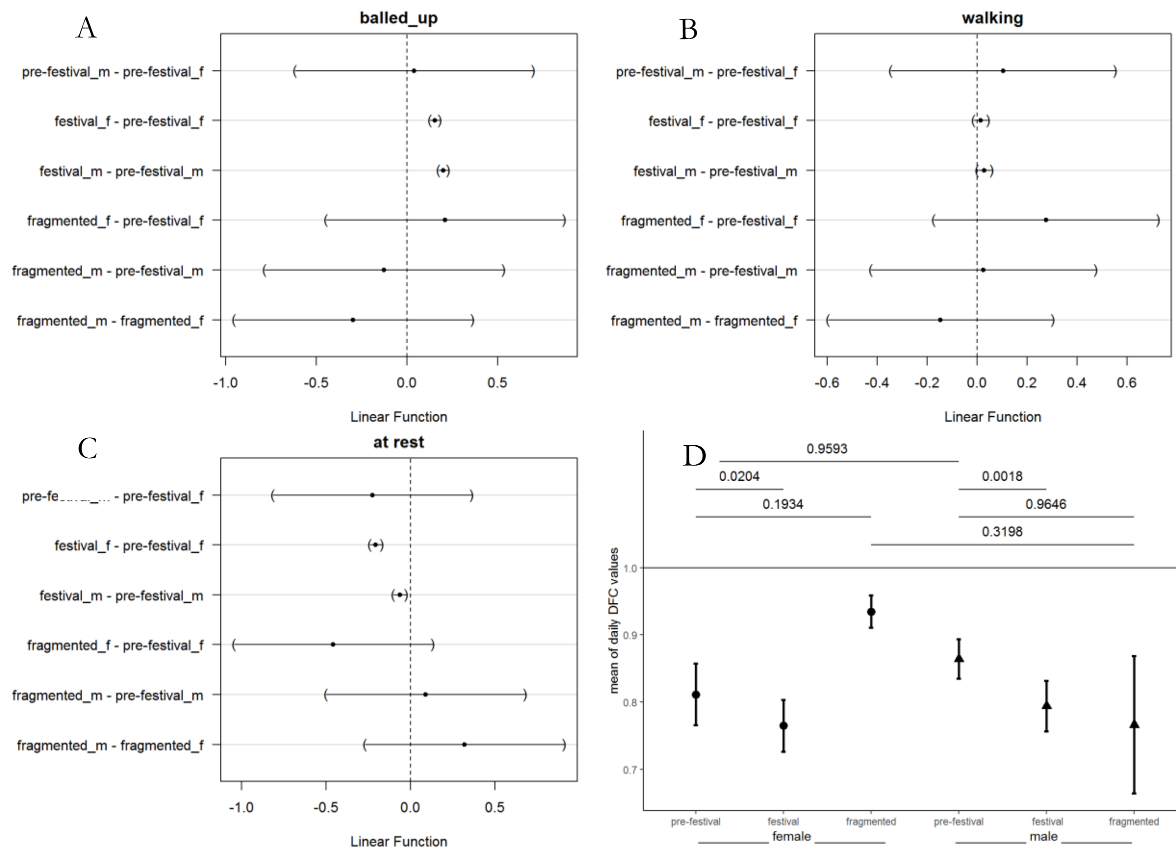


Figure 4.4: Behaviour and circadian rhythmic of hedgehogs in the context of acute/ transient and permanent habitat changes. A; odd value of balled up behaviour B; relative part of walking behaviour C; resting behaviour D; Degree of functional coupling; whiskers = 95% family-wise conf.int

Nesting Behaviour

During the pre-festival period, female hedgehogs used their nest in 66.1 % of the cases on more than one day. Nests of male hedgehogs were used with a probability of 57.8 % on the next day. Only the comparison within the dataset of 2016 (Treptower Park) pre-festival vs. festival showed differences between groups. During the festival, nests of male hedgehogs were used significantly shorter (Log-Rank, N=156, Mantel, p-Value = 0.02). Even the probability of using a new nest the next day is approx. 12 % lower (57.8 % vs. 45.5 %). With a probability of 0 %, a nest was used longer than eight days. In contrast for females, values were in general similar to or higher than during the pre-festival phase showing no significant differences (Log-Rank, N=88, Mantel, p-value=0.83, Figure 4.5).

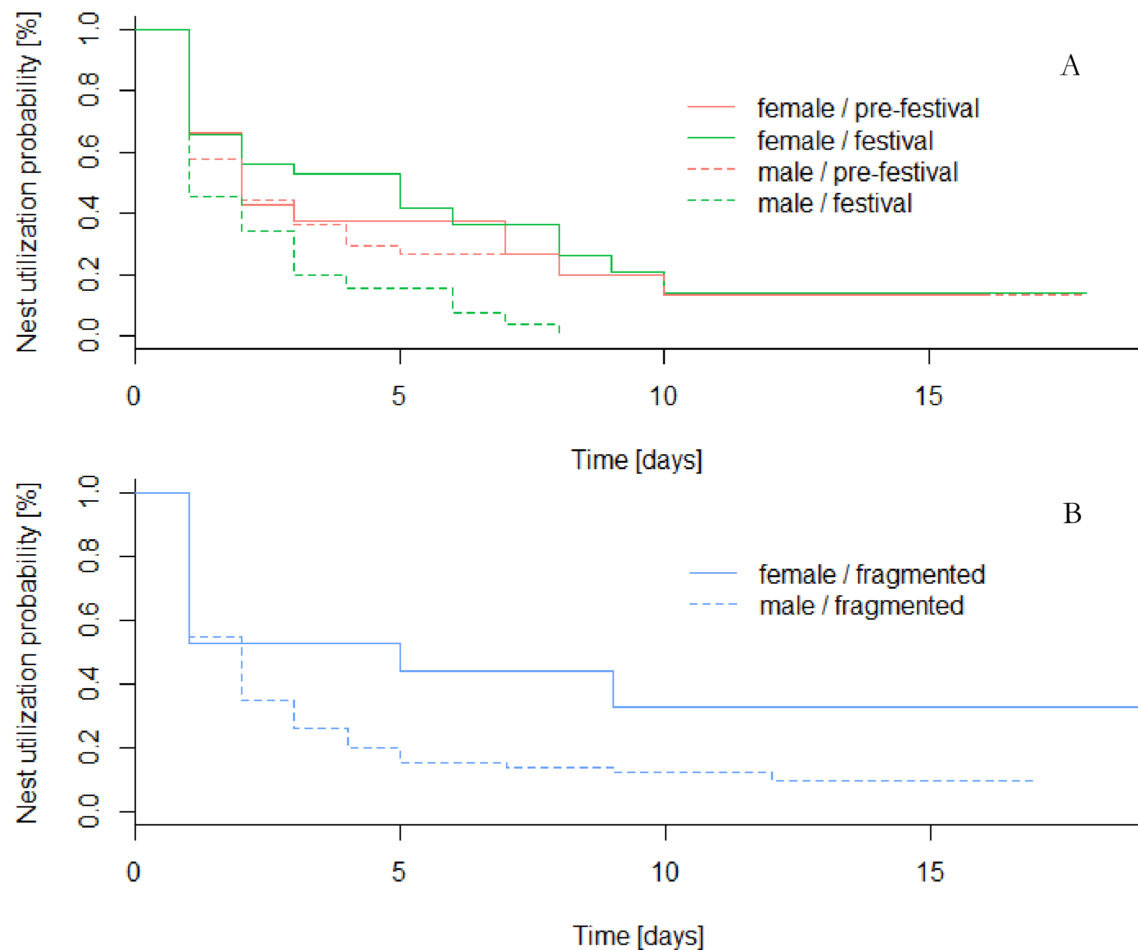


Figure 4.5: Nest utilization period probability of both datasets dataset A dataset B separately for sex. Dataset A: pre/after-festival and festival phase of 17 hedgehogs (nine males, eight females) p values coming from Log-rank test females: Log-Rank, N=88, Mantel, p-value=0.83; males: Log-Rank, N=156, Mantel, p-Value=0.02. Dataset B: fragmentation x-axis shorten for comparison.

4.5 Discussion

We showed various different crucial responses of hedgehogs to habitat change that could affect the survival of individuals. Consistent with our hypotheses, we found an influence of both stressors (fragmentation and transient change) on hedgehog space use, behaviour, circadian rhythm and nesting. Additionally, we showed differences of hedgehog responses to the two different stressors. With regard to the transient environmental change caused by the festival, both sexes decreased the size of the area utilized during the study, their movement speed and search intensity, while only females adjusted their space use in the fragmented habitat(1). Hedgehogs avoided the festival area (1) and changed their behaviour partly and modified their circadian rhythm (2, 3). The permanent, more static perturbation of fragmentation showed different adjustments (2, 3). These might be hints that hedgehogs can adjust to permanent stressors. It could also be possible that they behave in a maladaptive way, which could lead to negative fitness consequences. Surprisingly, stressors seemed to affect females differently than males. Males showed to be more active in avoiding or coping with the changes while females

seemed to be less able to cope with the changes although they were similarly affected (4). These findings could help to understand the role of hedgehogs and their future in the on-going urbanisation and if they are resilient to on-going declines in habitat space and increasing frequency of acute disturbances.

4.5.1 Movement Data

Area used

We found our results of area used nightly by hedgehogs reflect earlier findings on home ranges in residential areas (Dowding et al., 2010) and in other hedgehog species in suburban habitats (Schoenfeld and YOM-TOV, 1985), but are smaller than findings from other studies (compare Boitani and Reggiani, 1984; Kristiansson, 1984; Morris, 1988; Reeve, 1997; Rautio, Valtonen and Kunnasranta, 2013). It should be noted that in these studies which are contradicting to our results, a 100 % MCP was calculated, which is by definition bigger than our used 95% MCP and we used daily data while data from longer tracking periods was used in most these studies. Furthermore, it is important that home range size is not a constant value over time and earlier research showed large differences in individuals when comparing two following years (Dowding et al., 2010). We confirmed the general knowledge that males used larger areas than females (see Abu Baker et al., 2017). In our fragmented area, however, both sexes used at night a similar size of the area. This means that those individuals have higher energy consumption than their counterparts in less fragmented habitats. This could be especially important for the reproductive success of the females because the roamed area by the males was not bigger than of the individuals in our control. In a study on hedgehogs in rural habitats it was found that hedgehogs living further away from settlements had higher energy expenditure. The authors in the study concluded that this could be related to the longer distances hedgehogs have to cover (Pettett, Johnson, et al., 2017). In the same study, it was described that hedgehogs may restrict their movement in the presence of predators (badgers). It has to be evaluated if the trade-off between spatial fragmentation, high energy expenditure and advantages of close proximity to settlements and predator avoidance is changing (Pettett, Johnson, et al., 2017).

Speed and search intensity

Our reported values for movement speed seem low but since we included inactive and active data, this was no surprise and we report similar values to the mean speed of Ethiopian hedgehogs of 0.039 m/s (mean speed over sex and season). The differences are substantial enough to imply that hedgehogs in fragmented areas move on average 20 % times faster than in the unfragmented undisturbed habitat, a factor relevant for total energy turnover (Pettett,

Johnson, et al., 2017). In former studies, it was shown that hedgehogs move faster on larger streets, which could mean that hedgehogs on concrete paths like in the Tierpark also increase their speed if they use them (Bontadina, 1991). Commuting between foraging patches could be the reason for the higher speed in fragmented habitats, while the slow movement in hedgehogs is more related to foraging (Zingg, 1994; Braaker et al., 2014). This interaction could also lead to higher variance in the search intensity in the fragmented habitat. The higher values in search intensity during the festival could be a sign that hedgehog had to search for food more intensely on not optimal food patches or are related to the higher vigilance behaviour (lower speed) as response to new disturbances. While we find distributions that seem to be different and could show a general different behaviour within the urban matrix, having the active and inactive data of a relatively low number of individuals could influence the results of the turning angles. However, the movement of hedgehogs is influenced by anthropogenic disturbance and this is consistent with studies on other mammals, where a negative effect on long distance displacement was found (Tucker et al., 2018).

Shift

The detected shift in the area used nightly is a clear indication of the avoidance of the festival area. The open meadow which hedgehogs usually use to forage was blocked by visitors during as well as before and after the festival by workers. Hence, hedgehogs stayed longer, even up to the whole night, at the edge of the park. In some nights, they did not even leave the bushes in which their nests where located. The raw data suggest that although a shift of the used area seems to be normal from time to time. However, during the festival period hedgehogs avoid the former utilized areas (Supplementary Figure 4.5 and Supplementary Figure 4.6). Such a response was already shown for hedgehogs in farmland as a reaction to dramatic changes in resource quality (Doncaster and Krebs, 1993). In a similar semi-experimental approach, Koalas were before and during a music festival tracked and aversive changes in their behaviour detected (Phillips, 2016; FitzGibbon et al., 2017). In these situation it is crucial that space and escape routes are implemented to enable for animals the avoidance of the stressors.

4.5.2 Behaviour

Behavioural changes can be seen as an adjustment to a change, regardless of seasonal changes or during the ontogeny of an individual. Most of the time behavioural change and especially rapid changes can be a signal of disturbance (Berger, 2011). In our study, we observed a change in the hedgehogs curling-up or roll-up behaviour. Normally, this behaviour is a reaction to disturbance, even though it starts with spine erection and lead to a complete roll up if the animal is further

stressed (Reeve, 1994). In the interpretation of the data, we consider the possible misidentification between the resting and rolled up behaviour. Additionally, we have to consider the huge difference in the size of the 95 % confidence interval in the comparisons between two different individual groups (pre-festival vs. fragmented). It seems and was also expected that the comparison of different individual groups adds uncertainty to the analyses. For example, the mean proportion of identified rolled up behaviour of the females in the fragmented habitat was higher than the values from both the control and the festival (Supplementary Figure 4.8). However, only the comparison between the pre-festival and the festival was statistically different. In both cases, it could be possible that animals in the festival and the fragmented habitat encountered more situations that led to an increase of rolling-up behaviour. Visitors and overall noise of the festival as well as natural predators like foxes and badgers that roam in the fragmented habitat could cause it.

After the increase of the space used by females in the fragmented habitat an increase in walking was expected and measureable in female hedgehogs (however, statistically not significant). This again shows the need for a higher energetic investment when moving further distances (Pettett, Johnson, et al., 2017). Interestingly, in male hedgehogs, walking was slightly higher during the festival phase than in the pre-festival phase (mean ± conf.int 0.237 % ± 0.023 % vs. 0.241 % ± 0.022). This could indicate an active avoidance of the festival, maybe to avoid the festival (Supplementary Figure 4.9). A couple of male hedgehogs left the festival area during the festival and returned a few days later. Thus, the population signal could be influenced by a couple individuals. This confirms that hedgehogs move out of unfavourable habitats and thus, spatially avoid disturbances if necessary (Doncaster, Rondinini, and Johnson 2001).

The analyses of the DFC revealed the detailed analyses of the behaviour. This supports previous studies, which proved the DFC to be a tool to detect disturbance (Berger, 2011). This means that even in without behavioural validation of ACC data, old data can be analysed. As expected, in both sexes DFC decreased in the face of acute habitat change, showing a change in the internal rhythm of the animals, while there was no change detected in regard to the permanent (chronic) habitat change. Interestingly, we found the highest values in fragmented habitat changed in females (statistically not significant), which could mean that it is easy to maintain a regular activity in fragmented Tierpark and that the animals adjusted their biological rhythm. The fragmented values of the males had a higher variation displaying higher individual differences during this time of the year, which could be expected because male hedgehogs behave unpredictable during and shortly after the mating season (Rautio, Valtonen and Kunnasranta,

2013). In general, high values especially for nocturnal animals are expected and thus low DFC values indicate disturbance by stressors, disease but also parturition (Langbein, Scheibe and Eichhorn, 1998; Krone, Berger and Schulte, 2009). In our study some of the females gave birth to hoglets during the study period, this alone could influence the DFC. For a nocturnal animal like hedgehogs, a change in the circadian rhythm means exposure to even more stressors, which could lead to impairments of fitness. To verify DFC as tool to asses stress in wildlife a combination of energetic and hormonal assessment could give needed validation.

4.5.3 Nesting

Resting is important to recover and save energy. Hedgehogs either reuse or build a new nest to rest every day. This means more energy is invested into building a new nest instead of reusing a previously built nest. We recorded much higher turnovers of nests in our short periods from up to 40 days than rural hedgehogs in rural Irish populations (mean males 7.5 females 4.9 nest our study vs. both sexes 2.5; Haigh, O'Riordan and Butler, 2012), however, similar ranges to hedgehogs on a golf course in the suburbs of London (males 5 - 10 vs. 2 - 15, females 2 - 6 vs. 2 – 6; Reeve and Morris, 1985 Supplementary Table 4.10). Here, we show that females use less nesting sites compared to males in the same time frame and thus find the same results than previous studies (Reeve and Morris, 1985, Rautio, Valtonen and Kunnasranta, 2013). While we are lacking survival analyses of nesting studies, more data even of published projects with daily recordings could be reanalysed to find a baseline for European hedgehogs.

4.5.4 Conclusion and outlook

Overall, it is surprising that males reacted particularly stronger to the habitat change than females (nesting and behaviour). However, females had to care for hoglets which could impede their flexibility to react to the acute change, while males could move freely and thus, avoid the festival actively or facilitate the new circumstances. This individual behaviour enhances the behavioural flexibility of the population and should be approached if enough data is available (Hertel, Swenson and Bischof, 2017; Santini et al., 2018). Having the details of individual coping strategies can help to understand reactions to specific disturbances. In the anthropogenic changed environment, it is hard to observe the "true" behaviour of species. Some species adjust their behaviour to suboptimal habitats to avoid direct conflicts (cf. Refugee Species, Kerley, Kowalczyk and Cromsigt, 2012; Kuemmerle et al., 2012). For hedgehogs in the urbanized habitat it is possible that the observed behaviour (pre-festival) already is an adjusted suboptimal behaviour to urban environments, which on itself could lead to extinction. However, the shown reactions to the acute stress clearly indicate a disturbance which would worsen the situation. The

adjustment observed in the permanently fragmented habitat (higher speeds and increased area) of the females could facilitate adaptation to better and energetic more efficient locomotion (Wong and Candolin, 2015). The advanced technologies together with the biological understanding of species enable us to gain a deeper insight into the dynamics of ecosystems (Kays et al., 2015). The technical viewpoint and amount of data could bias the biological meaning easily. Thus it is important to combine close population monitoring and technological remote sensing data (Hebblewhite and Haydon, 2010; Shamoun-Baranes et al., 2012). The combination of GPS, ACC and behavioural data enabled us to understand the reaction and coping strategies of hedgehogs on a population level. It indicates behavioural flexibility, which could explain why an enigmatic old species like hedgehogs still exists, and that the appropriate management can help to recover the population numbers. We showed that small ground-dwelling animals can be used to disentangle the spatiotemporal influences of acute and permanent anthropogenic habitat changes. Our results show that humans and nature can share cities if space for natural dispersal of flora and fauna is ensured.

4.6 Acknowledgements

We would like to thank: Feona, Annika, Judith, Juline, Catrin, Dominik, Hanna Lea, Phillip, Selina, Martin, Sara, Lu, Lenz, Andres, Maria, Jennifer, Laurenz, Linda, Dana, Nadine, Lynn, Sharumathi, Saskia and Henrik for assistance during fieldwork. Special thank goes to Feona and Sara for preparing the nesting data for the analyses. We thank the Lollapalooza organising committee for allowing us to access the festival ground during the festival phase and the Tierpark Berlin for access to all areas. For the statistic, we thank Alexandre Courtiol who discussed with us the results and helped in some analyses. We also thank, and for their improvement of the manuscript. For proofreading and improvement of the manuscript, we have to thank Indra Möllenkotte, Shannon Currie, Tanja Straka and Sebastian Wantia.

Founding

This work was partly funded by the German Federal Ministry of Education and Research BMBF within the Collaborative Project "Bridging in Biodiversity Science - BIBS" (grant number 01LC1501A-H) and the Leibniz Institute for Zoo and Wildlife Research (IZW). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (IZW permit 2016-02-01) and German federal law (permission number Reg0115/15 and G0104/14).

4.7 References

Abu Baker, M. A., Reeve, N., Conkey, A. A. T., Macdonald, D. W. and Yamaguchi, N. (2017) 'Hedgehogs on the move: Testing the effects of land use change on home range size and movement patterns of free-ranging Ethiopian hedgehogs', PLoS ONE, 12(7), pp. 1–18. doi: 10.1371/journal.pone.0180826.

Baker, P. J. and Harris, S. (2007) 'Urban mammals: What does the future hold? An analysis of the factors affecting patterns of use of residential gardens in Great Britain', Mammal Review, 37(4), pp. 297–315. doi: 10.1111/j.1365-2907.2007.00102.x.

Banks, S. C., Piggott, M. P., Stow, A. J. and Taylor, A. C. (2007) 'Sex and sociality in a disconnected world: a review of the impacts of habitat fragmentation on animal social interactionsThis review is one of a series dealing with some aspects of the impact of habitat fragmentation on animals and plants. This series is ', Canadian Journal of Zoology, 85(10), pp. 1065–1079. doi: 10.1139/Z07-094.

Barthel, L. M. F., Hofer, H. and Berger, A. (2018) 'An easy, flexible solution to attach devices to hedgehogs (Erinaceus europaeus) enables long-term high-resolution studies', Ecology and Evolution, 9(1), pp. 672–679. doi: 10.1002/ece3.4794.

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015) 'Fitting Linear Mixed-Effects Models Using {lme4}', Journal of Statistical Software, 67(1), pp. 1–48. doi: 10.18637/jss.v067.i01.

Berger, A. (2011) 'Activity patterns, chronobiology and the assessment of stress and welfare in zoo and wild animals', International Zoo Yearbook, 45(1), pp. 80–90. doi: 10.1111/j.1748-1090.2010.00121.x.

Berger, A., Scheibe, K.-M., Michaelis, S. and Streich, W. J. (2003) 'Evaluation of living conditions of free-ranging animals by automated chronobiological analysis of behavior', Behavior Research Methods, Instruments, & Computers, 35(3), pp. 458–466. doi: 10.3758/BF03195524.

Berger, A., Scheibe, K. M., Eichhorn, K., Scheibe, A. and Streich, J. (1999) 'Diurnal and ultradian rhythms of behaviour in a mare group of Przewalski horse (*Equus ferus przewalskii*), measured through one year under semi-reserve conditions', Applied Animal Behaviour Science, 64(1), pp. 1–17. doi: 10.1016/S0168-1591(99)00026-X.

Bleicher, S. S. (2017) 'The landscape of fear conceptual framework: definition and review of current applications and misuses', PeerJ. PeerJ, Inc, 5, p. e3772. doi: 10.7717/peerj.3772.

Boitani, L. and Reggiani, G. (1984) 'Movements and activity patterns of Hedgehogs (*Erinaceus europaeus*) in Mediterranean coastal habitats', Zeitschrift Säugetierkunde, 49, pp. 193–206.

Bontadina, F. (1991) Strassenüberquerungen von Igeln Zürich, Instute of Zoology. University of Zurich.

Braaker, S., Moretti, M., Boesch, R., Ghazoul, J., Obrist, M. K. and Bontadina, F. (2014) 'Assessing habitat connectivity for ground-dwelling animals in an urban environment', Ecological Applications, 24(7), pp. 1583–1595. doi: 10.1890/13-1088.1.

Calenge, C. (2006) 'The package adehabitat for the R software: tool for the analysis of space and habitat use by animals', Ecological Modelling, 197, p. 1035.

Doncaster, C. P. (1994) 'Factors regulating local variations in abundance - field-tests on hedgehogs, erinaceus-europaeus', Oikos, 69(2), pp. 182–192. doi: 10.2307/3546136.

Doncaster, C. P. and Dickman, C. R. (1987) 'The ecology of small mammals in urban habitats. I. Population in a patchy environment', Journal of Animal Ecology, 56(2), pp. 629–640.

Doncaster, C. P. and Krebs, J. R. (1993) 'The wider countryside-principles underlying the responses of mammals to heterogeneous environments', Mammal Review. John Wiley & Sons, Ltd (10.1111), 23(3–4), pp. 113–120. doi: 10.1111/j.1365-2907.1993.tb00422.x.

Doncaster, C. P., Rondinini, C. and Johnson, P. C. D. (2001) 'Field test for environmental correlates of dispersal in hedgehogs *Erinaceus europaeus*', Journal of Animal Ecology, 70(1), pp. 33–46. doi: 10.1111/j.1365-2656.2001.00471.x.

Dowding, C. V., Harris, S., Poulton, S. and Baker, P. J. (2010) 'Nocturnal ranging behaviour of urban hedgehogs, *Erinaceus europaeus*, in relation to risk and reward', Animal Behaviour. Elsevier Ltd, 80(1), pp. 13–21. doi: 10.1016/j.anbehav.2010.04.007.

Fahrig, L. (2003) 'Effects of Habitat Fragmentation on Biodiversity', Annual Review of Ecology, Evolution, and Systematics, 34(1), pp. 487–515. doi: 10.1146/annurev.ecolsys.34.011802.132419.

FitzGibbon, S. I., Gillett, A. K., Barth, B. J., Taylor, B. and Ellis, W. A. (2017) 'Do koalas really get the blues? Critique of "Aversive behaviour by koalas (*Phascolarctos cinereus*) during the course of a music festival in northern New South Wales, Australia"", Australian Mammalogy. CSIRO PUBLISHING, 39(1), p. 108. doi: 10.1071/AM16016.

Fox, J. and Weisberg, S. (2011) An {R} Companion to Applied Regression. Second. Thousand Oaks {CA}: Sage. Available at: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

Haigh, A., O'Riordan, R. M. and Butler, F. (2012) 'Nesting behaviour and seasonal body mass changes in a rural Irish population of the Western hedgehog (Erinaceus europaeus)', Acta Theriologica, 57(4), pp. 321–331. doi: 10.1007/s13364-012-0080-2.

Hastings, A., Abbott, K. C., Cuddington, K., Francis, T., Gellner, G., Lai, Y. C., Morozov, A., Petrovskii, S., Scranton, K. and Zeeman, M. Lou (2018) 'Transient phenomena in ecology', Science, 361(6406). doi: 10.1126/science.aat6412.

Hebblewhite, M. and Haydon, D. T. (2010) 'Distinguishing technology from biology: a critical review of the use of GPS telemetry data in ecology.', Philosophical Transactions of the Royal Society of London. Series B, Biological sciences, 365(1550), pp. 2303–2312. doi: 10.1098/rstb.2010.0087.

Hertel, A. G., Steyaert, S. M. J. G., Zedrosser, A., Mysterud, A., Lodberg-Holm, H. K., Gelink, H. W., Kindberg, J. and Swenson, J. E. (2016) 'Bears and berries: species-specific selective foraging on a patchily distributed food resource in a human-altered landscape', Behavioral Ecology and Sociobiology. Behavioral Ecology and Sociobiology, 70(6), pp. 831–842. doi: 10.1007/s00265-016-2106-2.

Hertel, A. G., Swenson, J. E. and Bischof, R. (2017) 'A case for considering individual variation in diel activity patterns', Behavioral Ecology. Edited by A. Lindholm. Narnia, 28(6), pp. 1524–1531. doi:

10.1093/beheco/arx122.

Hijmans, R. J. (2017) 'geosphere: Spherical Trigonometry'. Available at: https://cran.r-project.org/package=geosphere.

Hofer, H. and East, M. L. (1998) 'Biological conservation and stress', Advances in the Study of Behavior, 21(1), pp. 405–525. doi: 10.1016/S0065-3454(08)60370-8.

Hothorn, T., Bretz, F. and Westfall, P. (2008) 'Simultaneous Inference in General Parametric Models', Biometrical Journal, 50(3), pp. 346–363.

Hubert, P., Julliard, R., Biagianti, S. and Poulle, M.-L. L. M. (2011) 'Ecological factors driving the higher hedgehog (Erinaceus europaeus) density in an urban area compared to the adjacent rural area', Landscape and Urban Planning. Elsevier B.V., 103(1), pp. 34–43. doi: 10.1016/j.landurbplan.2011.05.010.

Kays, R., Crofoot, M. C., Jetz, W. and Wikelski, M. (2015) 'Terrestrial animal tracking as an eye on life and planet', Science. American Association for the Advancement of Science, 348(6240), p. aaa2478. doi: 10.1126/science.aaa2478.

Kerley, G. I. H., Kowalczyk, R. and Cromsigt, J. P. G. M. (2012) 'Conservation implications of the refugee species concept and the European bison: King of the forest or refugee in a marginal habitat?', Ecography, 35(6), pp. 519–529. doi: 10.1111/j.1600-0587.2011.07146.x.

Krange, M. (2015) Change in the occurrence of the West European Hedgehog (Erinaceus europaeus) in western Sweden during 1950-2010. Karlstad University. Available at: http://kau.diva-portal.org/smash/record.jsf?pid=diva2%3A822331&dswid=9119 (Accessed: 5 December 2017).

Kristiansson, H. (1984) Ecology of a hedgehog Erinaceus europaeus population in southern Sweden. University of Lund, Sweden.

Krone, O., Berger, A. and Schulte, R. (2009) 'Recording movement and activity pattern of a White-tailed Sea Eagle (Haliaeetus albicilla) by a GPS datalogger', Journal of Ornithology. Springer-Verlag, 150(1), pp. 273–280. doi: 10.1007/s10336-008-0347-1.

Kuemmerle, T., Hickler, T., Olofsson, J., Schurgers, G. and Radeloff, V. C. (2012) 'Refugee species: Which historic baseline should inform conservation planning?', Diversity and Distributions, 18(12), pp. 1258–1261. doi: 10.1111/ddi.12013.

Langbein, J., Scheibe, K.-M. and Eichhorn, K. (1998) 'Investigations on periparturient behaviour in free-ranging mouflon sheep (*Ovis orientalis musimon*)', Journal of Zoology. Cambridge University Press, 244(4), pp. 553–561.

Lowry, H., Lill, A. and Wong, B. B. M. M. (2013) 'Behavioural responses of wildlife to urban environments', Biological Reviews. Blackwell Publishing Ltd, 88(3), pp. 537–549. doi: 10.1111/brv.12012.

Luniak, M. (2004) 'Synurbization–adaptation of animal wildlife to urban development', Proc. 4th Int. Symposium Urban Wildl. Conserv. …. Available at: https://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/snr0704-all.pdf#page=55

(Accessed: 4 March 2016).

McDonnell, M. J. and Hahs, A. K. (2015) 'Adaptation and Adaptedness of Organisms to Urban Environments', Annual Review of Ecology, Evolution, and Systematics. Annual Reviews, 46(1), pp. 261–280. doi: 10.1146/annurev-ecolsys-112414-054258.

Michelot, T., Langrock, R. and Patterson, T. A. (2016) 'moveHMM: an R package for the statistical modelling of animal movement data using hidden Markov models', Methods in Ecology and Evolution, 7(11), pp. 1308–1315. doi: 10.1111/2041-210X.12578.

Mori, E., Menchetti, M., Bertolino, S., Mazza, G. and Ancillotto, L. (2015) 'Reappraisal of an old cheap method for marking the European hedgehog', Mammal Research. Springer Berlin Heidelberg, 60(2), pp. 189–193. doi: 10.1007/s13364-014-0210-0.

Morris, P. A. (1988) 'A study of home range and movements in the hedgehog (*Erinaceus europaeus*)', Journal of Zoology. Blackwell Publishing Ltd, 214(3), pp. 433–449. doi: 10.1111/j.1469-7998.1988.tb03751.x.

Morris, P. A. (1997) Released, Rehabilitated Hedgehogs: A follow-up study in Jersey. Available at: http://wildpro.twycrosszoo.org/s/00ref/MiscellaneousContents/D151Hedgehog_DiscDocs03/J147_6_w1/J147_6_w1.htm (Accessed: 31 March 2016).

Pebesma, E. (2018) 'Simple Features for R: Standardized Support for Spatial Vector Data', The R Journal. Available at: https://journal.r-project.org/archive/2018/RJ-2018-009/index.html.

Pettett, C. E., Johnson, P. J., Moorhouse, T. P., Hambly, C., Speakman, J. R. and Macdonald, D. W. (2017) 'Daily energy expenditure in the face of predation: hedgehog energetics in rural landscapes', The Journal of Experimental Biology, 220(3), pp. 460–468. doi: 10.1242/jeb.150359.

Pettett, C. E., Moorhouse, T. P., Johnson, P. J. and Macdonald, D. W. (2017) 'Factors affecting hedgehog (Erinaceus europaeus) attraction to rural villages in arable landscapes', European Journal of Wildlife Research. European Journal of Wildlife Research, 63(3), p. 12. doi: 10.1007/s10344-017-1113-6.

Phillips, S. (2016) 'Aversive behaviour by koalas (*Phascolarctos cinereus*) during the course of a music festival in northern New South Wales, Australia', Australian Mammalogy. CSIRO PUBLISHING, 38(2), p. 158. doi: 10.1071/AM15006.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team (2018) '{nlme}: Linear and Nonlinear Mixed Effects Models'. Available at: https://cran.r-project.org/package=nlme.

R Core Team (2018) R: A language and environment for statistical computing., R Foundation for Statistical Computing, Vienna, Austria.

Rautio, A., Valtonen, A. and Kunnasranta, M. (2013) 'The effects of sex and season on home range in European hedgehogs at the northern edge of the species range', Annales Zoologici Fennici, 50(1–2), pp. 107–123. doi: 10.5735/086.050.0110.

Reeve, N. J. (1994) Hedgehogs. T & A. D Poyser, London.

Reeve, N. J. (1997) 'The Survival and Welfare of Hedgehogs (*Erinaceus europaeus*) After release back into the wild', Animal Welfare, 7, pp. 189–202.

Reeve, N. J. and Morris, P. A. (1985) 'Construction and use of summer nests by the hedgehog Erinaceus europaeus', Mammalia, 49(2), pp. 187–194.

Rousset, F. and Ferdy, J.-B. (2014) 'Testing environmental and genetic effects in the presence of spatial autocorrelation', Ecography, 37(8), pp. 781–790. Available at: http://dx.doi.org/10.1111/ecog.00566.

Rykiel, E. J. (1985) 'Towards a definition of disturbance', Australian Journal of Ecology, 10, pp. 361–365.

Santini, L., González-Suárez, M., Russo, D., Gonzalez-Voyer, A., von Hardenberg, A. and Ancillotto, L. (2018) 'One strategy does not fit all: determinants of urban adaptation in mammals', Ecology Letters. Edited by U. Brose. John Wiley & Sons, Ltd (10.1111), pp. 1–12. doi: 10.1111/ele.13199.

Scheibe, K. M., Berger, A., Langbein, J., Streich, W. J. and Eichhorn, K. (1999) 'Comparative Analysis of Ultradian and Circadian Behavioural Rhythms for Diagnosis of Biorhythmic State of Animals', Biological Rhythm Research, 30(2), pp. 216–233. doi: 10.1076/brhm.30.2.216.1420.

Schoenfeld, M. and YOM-TOV, Y. (1985) 'The biology of two species of hedgehog s, *Erinaceus europaeus concolor* and *Hemiechinus auritus aegyptius*, in Israel', Mammalia. Walter de Gruyter, Berlin / New York, 49(3), pp. 339–354. doi: 10.1515/mamm.1985.49.3.339.

Shamoun-Baranes, J., Bom, R., van Loon, E. E., Ens, B. J., Oosterbeek, K. and Bouten, W. (2012) 'From sensor data to animal behaviour: An oystercatcher example', PLoS ONE, 7(5), p. e37997. doi: 10.1371/journal.pone.0037997.

Shepard, D. B., Kuhns, A. R., Dreslik, M. J. and Phillips, C. A. (2008) 'Roads as barriers to animal movement in fragmented landscapes', Animal Conservation. John Wiley & Sons, Ltd (10.1111), 11(4), pp. 288–296. doi: 10.1111/j.1469-1795.2008.00183.x.

Sikes, R. S., Gannon, W. L., Committee, A. C. and U. and Animal Care and Use Committee (2011) 'Guidelines of the American Society of Mammalogists for the use of wild mammals in research', Journal of Mammalogy, 92(1), pp. 235–253. doi: 10.1644/10-MAMM-F-355.1.

Sinz, R. and Scheibe, K. (1976) 'Systemanalyse der multioszillatorischen Funktionsordnung im zirkadianen und ultradianen Frequenzbereich und ihr Indikationswert für Belastungswirkungen, dargestellt am Beispiel verschiedener Licht-Dunkel-Verhältnisse bei der Intensivhaltung von Schafen', Acta Biol Med Germ, 35(3–4), pp. 414–465.

Soanes, K., Taylor, A. C., Sunnucks, P., Vesk, P. A., Cesarini, S. and van der Ree, R. (2018) 'Evaluating the success of wildlife crossing structures using genetic approaches and an experimental design: Lessons from a gliding mammal', Journal of Applied Ecology. Edited by Y. Wiersma. Wiley/Blackwell (10.1111), 55(1), pp. 129–138. doi: 10.1111/1365-2664.12966.

Stillfried, M., Gras, P., Börner, K., Göritz, F., Painer, J., Röllig, K., Wenzler, M., Hofer, H., Ortmann, S. and Kramer-Schadt, S. (2017) 'Secrets of Success in a Landscape of Fear: Urban Wild Boar Adjust Risk Perception and Tolerate Disturbance', Frontiers in Ecology and Evolution. Frontiers, 5, p. 157. doi: 10.3389/fevo.2017.00157.

Therneau, T. M. (2015) 'A Package for Survival Analysis in S'. Available at: https://cran.r-

project.org/package=survival.

Tucker, M. A., Böhning-Gaese, K., Fagan, W. F., Fryxell, J. M., Van Moorter, B., Alberts, S. C., Ali, A. H., Allen, A. M., Attias, N., Avgar, T., Bartlam-Brooks, H., Bayarbaatar, B., Belant, J. L., Bertassoni, A., Beyer, D., Bidner, L., van Beest, F. M., Blake, S., Blaum, N., Bracis, C., Brown, D., de Bruyn, P. J. N., Cagnacci, F., Calabrese, J. M., Camilo-Alves, C., Chamaillé-Jammes, S., Chiaradia, A., Davidson, S. C., Dennis, T., DeStefano, S., Diefenbach, D., Douglas-Hamilton, I., Fennessy, J., Fichtel, C., Fiedler, W., Fischer, C., Fischhoff, I., Fleming, C. H., Ford, A. T., Fritz, S. A., Gehr, B., Goheen, J. R., Gurarie, E., Hebblewhite, M., Heurich, M., Hewison, A. J. M., Hof, C., Hurme, E., Isbell, L. A., Janssen, R., Jeltsch, F., Kaczensky, P., Kane, A., Kappeler, P. M., Kauffman, M., Kays, R., Kimuyu, D., Koch, F., Kranstauber, B., LaPoint, S., Leimgruber, P., Linnell, J. D. C., López-López, P., Markham, A. C., Mattisson, J., Medici, E. P., Mellone, U., Merrill, E., de Miranda Mourão, G., Morato, R. G., Morellet, N., Morrison, T. A., Díaz-Muñoz, S. L., Mysterud, A., Nandintsetseg, D., Nathan, R., Niamir, A., Odden, J., O'Hara, R. B., Oliveira-Santos, L. G. R., Olson, K. A., Patterson, B. D., Cunha de Paula, R., Pedrotti, L., Reineking, B., Rimmler, M., Rogers, T. L., Rolandsen, C. M., Rosenberry, C. S., Rubenstein, D. I., Safi, K., Saïd, S., Sapir, N., Sawyer, H., Schmidt, N. M., Selva, N., Sergiel, A., Shiilegdamba, E., Silva, J. P., Singh, N., Solberg, E. J., Spiegel, O., Strand, O., Sundaresan, S., Ullmann, W., Voigt, U., Wall, J., Wattles, D., Wikelski, M., Wilmers, C. C., Wilson, J. W., Wittemyer, G., Zięba, F., Zwijacz-Kozica, T. and Mueller, T. (2018) 'Moving in the Anthropocene: Global reductions in terrestrial mammalian movements', Science, 359(6374), pp. 466–469. doi: 10.1126/science.aam9712.

Walker, L. R. (2012) The biology of disturbed habitats. Oxford, UK: Oxford University Press.

Wilcove, D. S. D., McLellan, C. H. C., Biology, A. D.-C., 1986, U. and Dobson, A. P. (1986) 'Habitat fragmentation in the temperate zone.', in Conservation biology 6, pp. 237–256.

Wong, B. B. M. and Candolin, U. (2015) 'Behavioral responses to changing environments', Behavioral Ecology. Oxford University Press, 26(3), pp. 665–673. doi: 10.1093/beheco/aru183.

Zingg, R. (1994) Aktivitaet Habitat- und Raumnutzung von Igeln (Erinaceus europaeus) in einem ländlichen Siedlungsgebiet. Universität Zürich.

Figure 4.6: Hedgehog squeezing under the VIP Container of the Lollapalooza festival

5 Music festival makes hedgehogs move: How individuals cope behaviourally in response to human-induced stressors

Submitted in March 2019 to Animals to the Special Issue 'Behaviour and Management of Urban Wildlife

Now published

Wanja Rast [1, §], Leon M.F. Barthel[1, 2, §] and Anne Berger [1,2,*]

[1]Leibniz-Institute for Zoo- and Wildlife Research (IZW), Department Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany; rast@izw-berlin.de (W.R.); barthel@izw-berlin.de (L.B.)
[2]Berlin Brandenburg Institute of Advanced Biodiversity Research (BBIB), Berlin, Germany; https://www.bbib.org
*berger@izw-berlin.de; phone: +49-030-5168328
§ These authors contributed equally to this work

Simple Summary: 'Mega-events' like concerts or festivals can still impact wildlife even when protective measures are taken. We remotely observed eight hedgehogs in a Berlin city park before and during a music festival using measuring devices attached to their bodies. While the actual festival only lasted two days (with about 70,000 visitors each day), setting the area up and removing the stages and stalls took 17 days in total. Construction work continued around the clock causing an increase in light, noise and human presence throughout the night. In response, the hedgehogs showed clear changes in their behaviour in comparison to a 19 day period just before the festival. We found, however, that different individuals responded differently to these changes in their environment. This individuality and behavioural flexibility could be one reason why hedgehogs are able to live in big cities.

Abstract: Understanding the impact of human activities on wildlife behaviour and fitness can inform their sustainable management. We wanted to identify behavioural responses to anthropogenic stress in an urban species during a semi-experimental field study. We equipped eight urban hedgehogs (*Erinaceus europaeus*; four per sex) with biologgers to record their behaviour before and during a mega music festival (2 x 19 days) in Treptower Park, Berlin. We used GPS to monitor spatial behaviour, the VHF-loggers to quantify daily nest utilisation, and accelerometers to distinguish between different behaviours at a high resolution and calculate daily disturbance (using Degrees of Functional Coupling). The hedgehogs showed clear behavioural differences between the pre-festival and festival phases. We found evidence supporting highly individual strategies, varying between spatial and temporal evasion of the disturbance. Averaging the responses of the individual animals or only examining one behavioural parameter masked these potentially different individual coping strategies. Using a combination of different minimally-invasive biologger types, we were able to show a high inter-

individual behavioural variance of urban hedgehogs resulting from an anthropogenic disturbance. Such behavioural responses might be a precondition to persist successfully in urban environments.

Keywords: accelerometer, anthropogenic disturbance, behavioural flexibility, behaviour recognition, Erinaceae, non-invasive stress detection, ODBA, urban wildlife

5.1 Introduction

Human activity has a significant impact on biological diversity and the persistence of wildlife populations [1]. One example of such human activity is the global process of urbanization, which leads to fast and drastic environmental changes for wildlife [2–4]. Some species avoid urbanized landscapes while others thrive and persist in them [5]. Urban areas are not only characterized by an altered landscape but are also hotspots of human activity that may constrain the behavioural repertoire of urban wildlife [6]. A detailed understanding of how human activity impacts urban wildlife populations is essential for conservation and wildlife management and for the resolution of human-wildlife-conflicts.

Studying wildlife responses to human disturbance under standardized lab-conditions allows inference about causality but often lacks ecological realism. Natural conditions and the complexity of ecological processes are difficult, if not impossible, to integrate into lab-studies [7]. In comparison, experimental field studies include a high level of ecological realism, but often cannot identify causality and face many methodical challenges. One challenge is being able to recognize, understand, and clearly distinguish the various environmental factors that affect animal behaviour. Often, there will be a complex set of ecological relationships which cannot be controlled by the researcher. However, recent advances in biologging, to remotely monitor animal behaviour and physiology, have removed many of the former limitations of field studies. It is now possible to record the behaviour of free-living wild animals by logging them with high-resolution 3D accelerometers. These data can be used to distinguish different behaviours, as well as to evaluate the rhythmic structure of behaviours.

Behavioural rhythms have evolved as adaptations to the environment and enable organisms to be active at the times most suited to their physiology or ecology. Aberrations from these patterns can result in impairments of fitness. In this study, we focus on a particularly important rhythmic structure, the circadian rhythmicity of activity. Analysis of circadian rhythmicity of activity can be measured using the Degree of Functional Coupling (DFC) which can indicate de-synchronisation of general behaviour patterns caused by stress, illness or disturbances [8,9], and so is well suited to understand the impacts of human activity on urban wildlife.

The European hedgehog (*Erinaceus europaeus*) is protected in many parts of Europe but has still experienced serious and continuous declines in the last decades [10,11]. Although hedgehogs originally inhabited rural mosaic structures, they now have higher population densities in urban areas [11,12]. For the protection and management of this species, it is therefore important to assess the adaptive capacity and limits of hedgehogs to urban conditions.

We measured the effects of a music festival in a city park on the behaviour of urban hedgehogs to investigate the hedgehog's side of this human-wildlife-conflict. The biology of the hedgehog can make behavioural studies difficult [13]. They are nocturnal, small and keep hidden during the day, which makes the use of animal-borne loggers all the more valuable. In this study, we work with easy-to-use, non-invasive loggers to infer the stress responses of animals (which are usually estimated by physiological data) by estimating their behaviour changes to a serious stressor through the combination of different loggers and differentiated analysis of the measured data.

We recorded the spatiotemporal behaviour of hedgehogs and analysed it on a fine temporal scale (minutes), before and during a large festival. A music festival in the city is not only a site of human activity but also creates a sudden and drastic change in the environment. Because the festival site was never previously used for such an event it is unlikely that the hedgehogs would be accustomed to such a disturbance. We hypothesise that hedgehogs change their spatial-temporal behaviour in response to the festival event. However, behavioural responses are often individual- and sex-specific which should be considered when studying the effects of anthropogenic disturbances [14,15]. As hedgehogs may adjust their behaviour to avoid contact with human disturbance we predict a general decrease in the area used nightly and DFC during the festival but individual responses in behaviour and activity may vary.

5.2 Materials and Methods

5.2.1 Study Area

Fieldwork was conducted from July to September 2016 within an urban park (Treptower Park) of 88.2 ha, in southeast Berlin, Germany (52.48846°N, 13.46974°E). Treptower Park is open to the general public and contains lawns of short grass, variable shrub density, gravel footpaths, a playground and a monument site. The park is surrounded by urban pedestrian areas, tarmacked streets and parking areas to the east and south and is bounded by the river Spree to the north and a railway embankment to the west. Within this urban park, streets, fences and the railway embankment create obstacles to hedgehog movement.

5.2.2 Semi-experimental design

We defined the pre-festival phase as the 10th of August until the 28th of August; while the festival phase lasted from the 29th of August until the 16th of September. Festival phase consisted of three phases: construction, the actual festival and deconstruction. The actual festival, with about 140,000 visitors, took place on the 10th and 11th of September. The construction of the festival started on the 29th of August and the deconstruction concluded on the 16th of September. In total there were 19 days for the pre-festival phase and 19 days for the festival phase.

During the construction phase, the whole area was fenced and big mats of aluminium and rubber were placed throughout the park to allow trucks to drive in. Several stands and the two main stages were built in our sample area which was a 16 ha section of the whole park. The festival ground extended to other parts of the park which were separated from our sampling area by a major four-lane road, which was out of use during the festival. All bushes were fenced to protect wildlife from the festival visitors during the festival phase. During the actual festival, from 10:00 am to midnight, visitors could enter the festival area and music was played from 10:30 am to 23:00 pm on different stages accompanied by light shows. Immediately after the end of the actual festival event, the deconstruction of all fences, stages and mats started.

Table 5.1: Animal identification number (ID), sex and body mass (at the date of logger attachment) of the studied hedgehogs

Animal ID	Sex	Body mass [g]
01_2016	m	1060
02_2016	f	1090
08_2016	f	795
09_2016	m	830
13_2016	f	725
17_2016	f	1480
19_2016	m	890
21_2016	m	1015

5.2.3 Study animals and logger attachment

At the beginning of August 2016, we carried out two-night surveys at least one hour after sunset to find active hedgehogs by spotlighting (P14.2, LED Lenser, Solingen, Germany). Each hedgehog was marked with five yellow shrink-fit plastic tubes on the spines [16]. The tubes were numbered to allow individual identification during recapture [17].

We equipped 17 hedgehogs with VHF transmitters. On the 9th of August, we selected eight out of the 17 hedgehogs (four of each sex) and also equipped them with GPS/ACC loggers (E-obs GmbH, München, Germany) using a backplate system described by [18]. We only used

hedgehogs with the required body mass of 600g to meet the recommended 5 % body mass rule recommended by [19] (Table 1).

Once every week during the study, we weighed all hedgehogs and inspected them for any health problems. On these occasions, we also recharged the data loggers. Nesting behaviour was recorded every day by locating the VHF signals of each of the 17 VHF-logged hedgehogs (TRX-1000S, Wildlife Materials Inc., Murphysboro, IL, USA, or Wide Range Receiver AR 8200, AOR Ltd., Tokyo, Japan). On the 20th of September, we removed all loggers, VHF transmitters and back plates.

5.2.4 Logger setup

GPS positions were taken from 7:00 pm to 7:00 am in 5 min intervals, with bursts of five points to increase accuracy. The VHF transmitters sent signals continuously throughout the whole study period. Acceleration data was recorded alongside the GPS data by the e-obs tags. These accelerometers were programmed to record a short burst of high-resolution data. We chose a sampling frequency of 100 Hz per axis for the present study. All three available axes were measured simultaneously. For individuals 01_2016 and 19_2016 a burst was 2.64 s long resulting in 264 measurements per axis. All other individuals were recorded with 2.5 s long bursts with 250 measurements per axis. The burst length is only important for three of the 25 calculated predictors used for the model (see 'Behavioural prediction and budget' below). Bursts were recorded every minute.

To account for missing data caused by power loss or logger malfunctioning we removed all dates with less than 1430 (1440 for complete 24 h) measurements between 0:00 am and 11:59 pm from the data set. This ensured that only days with the same amount of recordings during both day and night were included, which avoided any bias towards behaviours that only occurs during a specific time of day.

5.2.5 Data Analysis

Spatial data analysis

Because of large fluctuations in the co-ordinates of some GPS points, we excluded all points that were more than 1000 m away from the study site. We then calculated the average of all remaining points per time event. Outliers of more than 2 m/s speed from one location to the next location were excluded. We grouped the remaining points for each night to calculate the used areas from the evening of one day to the next morning.

We calculated used areas for each night and both phases, pre-festival and festival. We computed the Kernel density estimation 50 % (KDE50) for each phase separately using the functions of the adhabitatHR package [20] in R [21] (version 3.5.1, R Core Team, 2018) and R Studio [22]. The KDE50 is used to evaluate the core area used by the hedgehogs per night.

We monitored day nests for all 17 radio-tagged hedgehogs (nine males, eight females, including the individuals from Table 1) from 10th of August until 21st of September (five days after the festival). We calculated the nest utilization (survival Kaplan-Meier-method) period probability using R (package 'survival'); we then used the log rank test (Mantel method, package 'coin') to test the equality of the utilization period distributions between non-festival (before and after) and festival phase for each sex separately [23].

Acceleration data analyses

Behavioural prediction and budget

For behavioural prediction, we used a supervised machine learning algorithm which uses data of known behaviour to train and test the model. We took the data of known behaviours used for the model from a pre-study. In this pre-study, hedgehogs were logged using the same protocol as the present study and observed in June and July 2016 over several nights, using six hedgehogs (three females, three males) from the same study area in the Treptower Park. In total four behaviours were considered for the analysis of these animals: resting, defined as not moving regardless of the body posture; rolling up, defined as curling-up in defense to make a tight ball; walking, defined as slow locomotion; and running, defined as fast locomotion [24].

The data in this pre-study were recorded in bursts of 2.64 s length with 100 Hz for each of the three axes resulting in 264 data per burst and axis. To build the model, all six individuals were pooled into one data set. The model is based on summary statistics calculated from the raw acceleration data using the package accelerateR [25]. For this model, we computed the following set of predictors: mean, standard deviation, inverse coefficient of variation, weighted mean of the autocorrelated power spectrum, variance, kurtosis and skewness all for each axis separately, and, from a combination of all three axes: q [26], pitch, roll [27] and overall dynamic body acceleration (ODBA) [28].

We choose the Support Vector Machine (SVM) algorithm to classify the predictors for each burst. The SVM represents the predictors in a multi-dimensional space. To separate data points of different classes from each other, a hyperplane is constructed between points of two classes. Points are then classified according to their relative position to the hyperplane [29]. This method

was designed to work with binary data. By joining multiple SVMs it is possible to work with data that have more than two classes [30]. In the present study, we considered three classes: resting, balling up and locomotion. To account for behaviours that are not included in the model but might occur in hedgehogs, a threshold was set for the SVM. A prediction was only considered reliable when the probability of belonging to a class exceeded 0.7. Otherwise, the behaviour was classified as 'other' behaviour.

The recall (True positives / (True positives + False negatives)) and precision (True positives / (True positives + False positives)) [31] were calculated as well as the proportion of predictions that were classified as 'other' to evaluate the model after a leave one out cross validation. We used the package „e1071' [32] for the implementation in R.

We prepared the data for the animals of the present study in the same way as data from the model hedgehogs. In addition, we tested the raw data for missing measurements within the bursts. We removed all bursts where less data was recorded than expected under the burst settings. We then used the SVM model to assign a behaviour prediction to every burst and its corresponding timestamp. The probability threshold of 0.7 was used to assign the behaviour 'other' to all bursts that did not exceed the threshold.

We considered the behaviour of every individual for the pre-festival and festival phases separately. To test for changes in behaviour, we compared the proportion of every behaviour class between the pre-festival and festival phase with a Fisher's exact test with the fisher.test() function in R.

Daily activity pattern

We calculated the accumulated standard deviation (aSD) by summing up the standard deviation from all three axes for every burst. Using aSD, we calculated the index of diurnality (DI) based on the relative level of activity during daylight compared to night-time for each individual on a given day, with day starting at civil dawn. We used DI after [33] in which the different time spans of day or night are taken into account. DI ranges between -1 (absolutely nocturnal) and 1 (absolutely diurnal). We defined civil dawn and civil dusk as the border between day and night; date-specific times for civil twilight were obtained from the National Oceanic & Atmospheric Administration (NOAA, www.esrl.noaa.gov).

Restless phases during the day, such as those triggered by loud music during the festival, would increase the proportion of daytime activity and the DI would thus give an incomplete picture of the influence of the festival on the activity pattern of the hedgehogs. We, therefore, calculated

the time span between activity onset and civil dusk (TSdusk). The mean of the aSD was used as a threshold to distinguish between generally active (> mean aSD) and passive (< mean aSD) behaviour.

Overall dynamic body acceleration (ODBA)

The ODBA was introduced as a proxy for energy expenditure [34,35]. At its basis, it is a measurement of general animal body movement irrespective of behaviour. It was used to map the activity of the animals in order to compare the general activity between the two phases. The ODBA values were taken from the SVM model. To represent whole days the ODBA values for every 24 hour period (0:00 am - 11:59 pm) were summed up separately for every individual.

Stress detection

Degree of Functional Coupling (DFC) is a parameter to measure the synchrony of (internal) cyclic behaviour and the (external) environmental 24 h period, expressed with a value between 0 (no synchrony) and 1 (maximal synchronized) [8].

We used the aSD to calculate DFCs (see section Daily activity pattern). Following the protocol of Berger [8] et al. (2003), the time series was auto-correlated in order to filter out noise and enhance rhythmic components and, after a Fourier transform, was used to break the time series down into its rhythmic components described by the percentage of each component in the original time series. The longest Fourier period tested covers the entire length of the auto-correlation function (here three days); the shortest Fourier period tested is twice the sampling interval (here 2 minutes). The Degree of Functional Coupling is calculated by dividing the Fourier transformation components that harmonize with the 24-hour rhythm by the entirety of the Fourier spectrum. To gain an adequate statistical power of the 24 hour period, DFCs were calculated for time series of three days using a moving average (first data set covers day 1 to 3, second data set covers day 2 to 4, and so on). The resulting DFCs were assigned to the last day of the moving average (day 3 for the first set, day 4 for the second set, and so on).

Statistical analysis

We compared the area nightly used, DI, TSdusk, ODBA and DFC between the pre-festival and the festival phases with a Wilcoxon rank sum test using the wilcoxon.test() function in R. Statistical comparisons for nest utilisation and behaviour budget are explained in more detail in the respective sections.

5.3 Results

5.3.1 Spatial Results

The size of mean area nightly used (measured with KDE50) decreased in all eight hedgehogs during the festival phase. The Wilcoxon rank sum test calculated for each individual showed that differences in five out of eight hedgehogs were significant (08_2016, 09_2016, 17_2016, 19_2016, 21_2016)(Figure 5.1).

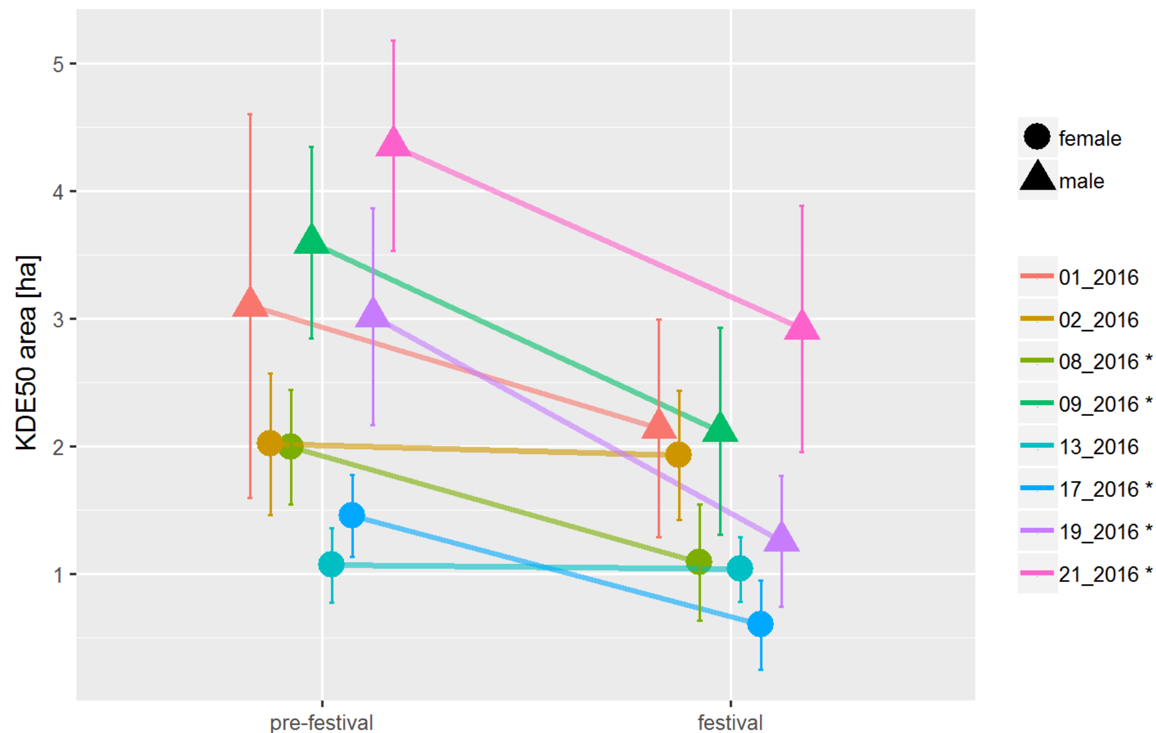


Figure 5.1: mean values with a confidence interval for nightly Kernel density estimation 50 % (50KDE) areas of eight hedgehogs in the pre-festival and festival phase (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour.

During the pre-festival phase, the probability of a nest being re-used the next day was 66.1 % for females and 57.8 % for males. During the festival, nests of male hedgehogs were used for a significantly shorter time (Log-Rank, N = 156, Mantel, Z = -2.3327, p-Value = 0.02). The probability of using a new nest was ~12 % lower in males (57.8 % vs. 45.5 %). No nests were used for longer than eight days. Values for females were similar in both phases. Differences were however not significant for any individual (Log-Rank, N = 88, Mantel, Z = 0.49502, p-value = 0.62, Figure 5.2).

Daily nest checks showed that individuals 02_2016 and 13_2016 gave birth between 22nd of August and the 28th of August and 29th of August and the 4th of September respectively.

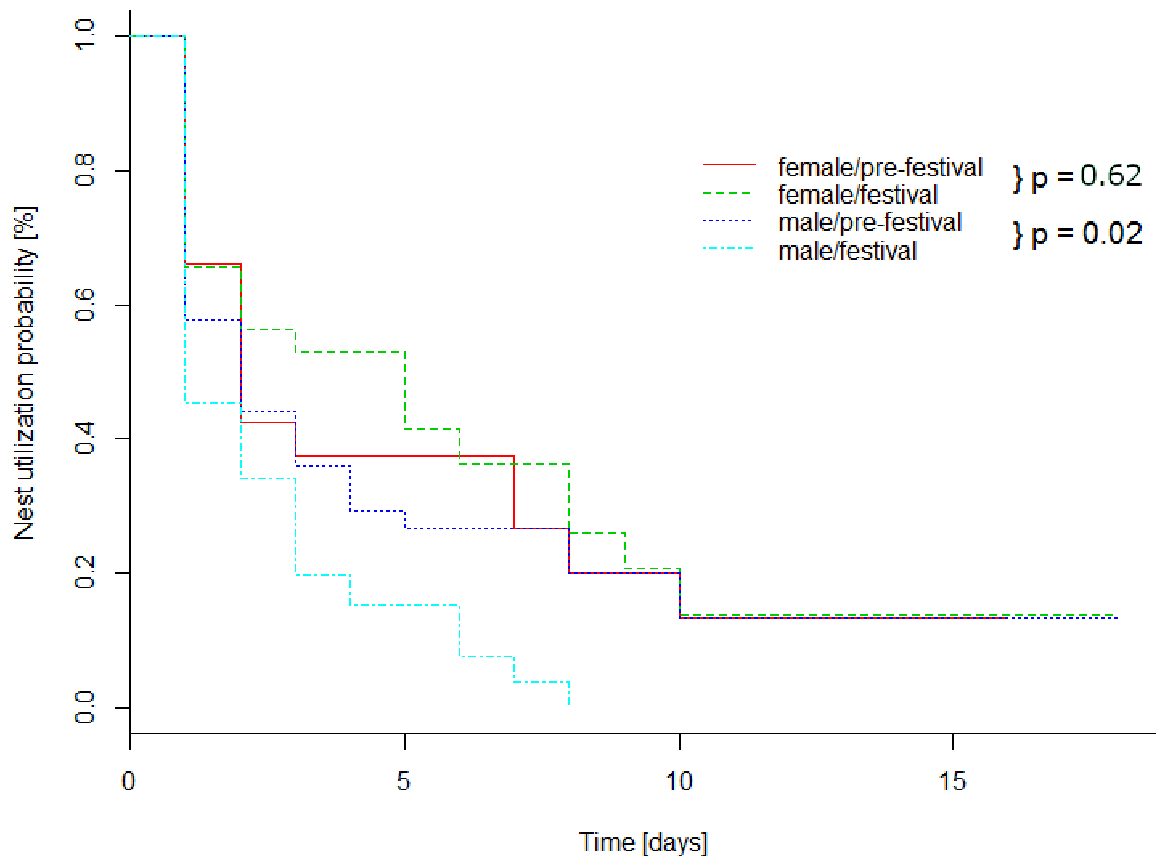


Figure 5.2: Nest utilization period probability of females and males during pre/after-festival and festival phase of 17 hedgehogs (nine males, eight females) p values coming from Log-rank test females: Log-Rank, N = 88, Mantel, p-value = 0.83; males: Log-Rank, N = 156, Mantel, p-Value = 0.02.

5.3.2 Model evaluation for behaviour prediction

The hedgehog behaviour model considered three different behaviours: immobile, balling up, and locomotion. The final data set consisted of 197 bursts for each of the three behaviour classes. The leave one out cross validation showed high values for recall and precision (Table 5.2). A total of 73 (12 %) of the 591 bursts were classified as 'other' behaviours due to the probability threshold of 0.7.

Table 5.2: Recall and precision [31] of the hedgehog model

	Recall	Precision
Immobile	0.77	0.88
Balling up	0.78	0.90
Locomotion	0.91	0.93

5.3.3 Behaviour prediction

Burst with missing data occurred only in individual 13_2016 where we removed a total of nine bursts. A programming error for the tag on individual 02_2016 led to the removal of five days of data at the beginning of the study (10th of August 2016 - 14th of August 2016). Finally we

removed a total of three days from the pre-festival phase for all hedgehogs except individuals 02_2016 (nine days) and 19_2016 (four days). The number of removed days for the festival phase ranged between 3 and 6 days. The tag of 09_2016 broke down after the 12th of September 2016 and therefore we removed this individual completely from the analysis as there are only three days with more than 1430 recordings per 24 hours of recording in the festival phase.

5.3.4 Behaviour budget

Hedgehog behaviour differed between the two phases of the festival (Table 5.3). Odds ratios range from 0.512548 to 1.814369. Immobile behaviour decreased in four individuals while three individuals showed no difference. Balling up increased in all but two individuals. Locomotion did not change for all the males while two females increased and two decreased locomotive behaviour. Because of the nature of the behaviour class ‘other’, an interpretation of these behaviours is not possible. For the sake of completeness, it ‘other’ behaviour will be reported but not discussed further.

Table 5.3: Changes in the behaviour during the festival phase in comparison to the pre-festival phase. Values represent the odds ratio for every behaviour separately with the corresponding p-value calculated with the Fisher's Exact Test. Odds ratios smaller than 1 indicate a decrease in the behaviour in the festival phase while an odds ratio greater than 1 indicate an increase.

Animal ID	Immobile	Balling up	Locomotion	Other
01_2016	0.808 $p = 2.337e-15$	1.47 $p < 2.2e-16$	0.972 $p = 0.2048$	0.862 $p = 8.251e-15$
02_2016	0.956 $p = 0.1839$	0.513 $p < 2.2e-16$	1.366 $p < 2.2e-16$	1.44 $p < 2.2e-16$
08_2016	0.623 $p < 2.2e-16$	1.367 $p < 2.2e-16$	0.863 $p = 3.896e-09$	1.027 $p = 0.1969$
13_2016	0.963 $p = 0.1525$	0.918 $p = 0.0002304$	1.652 $p < 2.2e-16$	0.768 $p < 2.2e-16$
17_2016	0.793 $p < 2.816e-15$	1.814 $p < 2.2e-16$	0.676 $p < 2.2e-16$	0.841 $p < 2.2e-16$
19_2016	0.847 $p = 1.254e-06$	1.238 $p < 2.2e-16$	0.969 $p = 0.1972$	0.855 $p = 5.468e-14$
21_2016	1.003 $p = 0.9202$	1.066 $p = 0.001328$	1.002 $p = 0.939$	0.933 $p = 0.000685$

5.3.5 Daily activity pattern

All studied hedgehogs were strictly nocturnal showing negative diurnality indices. Six out of eight hedgehogs reduced their DI and two animals (17_2016 and 19_2019) showed an increased DI

during the festival. Only one hedgehog showed a significant decrease (Wilcoxon test: W = 108 p-value = 0.04699) (09_2016) (Figure 5.3).

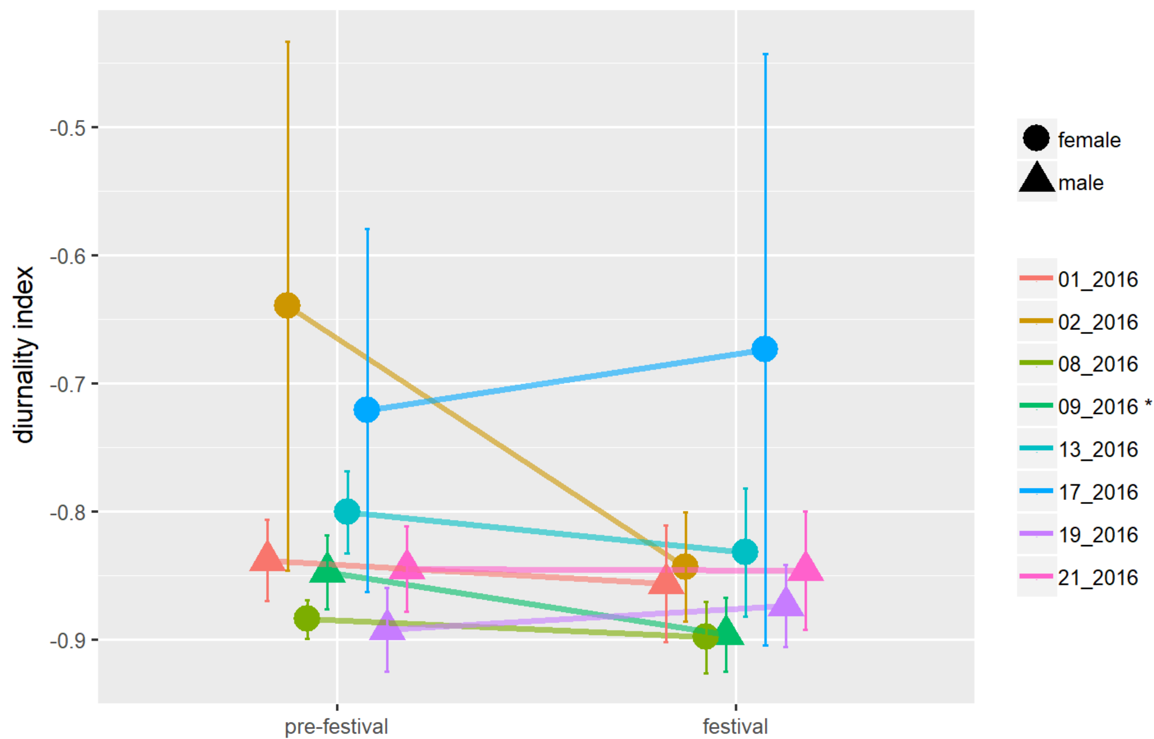


Figure 5.3: Mean values with confidence interval for the diurnality index DI of eight hedgehogs in the pre-festival and festival timeframe (significant differences are marked by *, Wilcoxon test cf. Supplementary Table 5.1). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour.

During the festival phase, all hedgehogs shifted their activity onset to a later time compared to the pre-festival phase, shown by the increased TSdusk (zero marks the time of civil dusk, negative TSdusk values represent time before civil dusk, positive TSdusk values represent time after civil dusk). For five out of eight hedgehogs (01_2016, 08_2016, 13_2016, 19_2016, 21_2016) these differences are significant, indicated by a Wilcoxon rank sum (Figure 5.4).

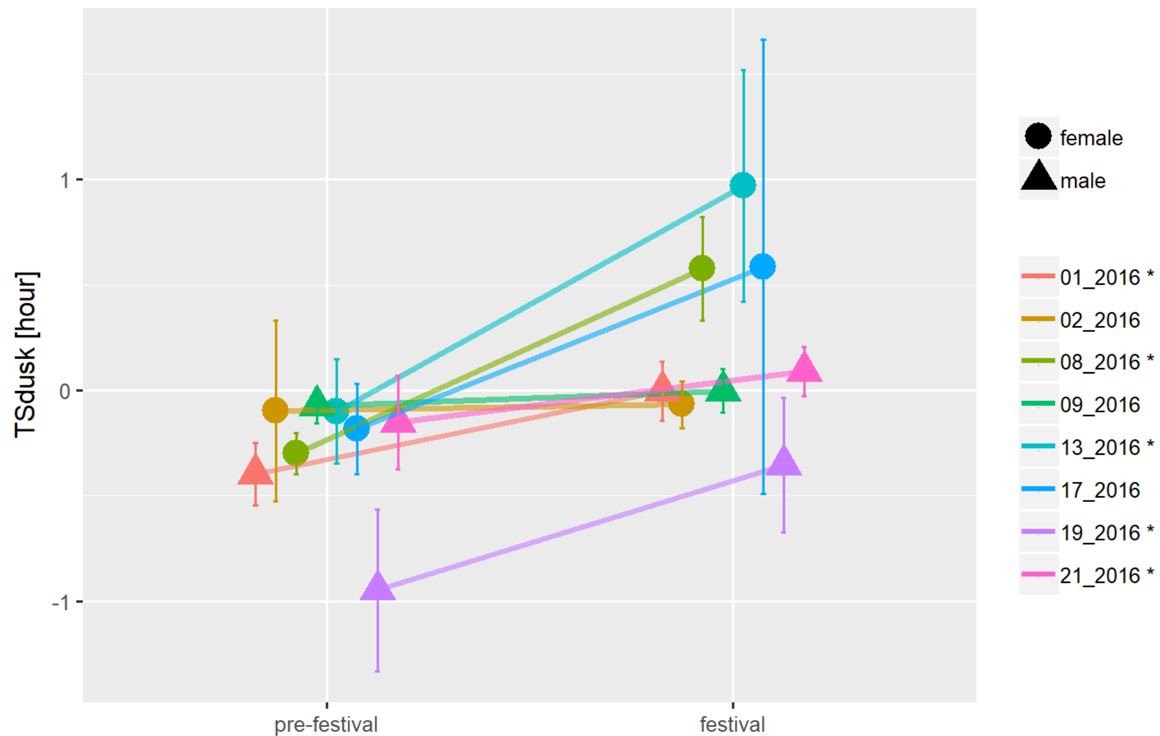


Figure 5.4: Mean values with confidence interval for the time span between activity onset and civil dusk (TSdusk) of eight hedgehogs in the pre-festival and festival timeframe (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour.

5.3.6 ODBA analysis

Mean daily ODBA values were similar between the pre-festival and festival phase for most hedgehogs (Figure 5.5). Two individuals (08_2016, 17_2016) show a significant difference (Wilcoxon test: W 0 174 p-value = 0.001498, W = 162 p-value = 0.01007). Both individuals showed lower mean daily ODBA during the festival compared to the pre-festival phase. Individual 09_2016 was also removed from the ODBA analysis for reasons explained in section behaviour analysis.

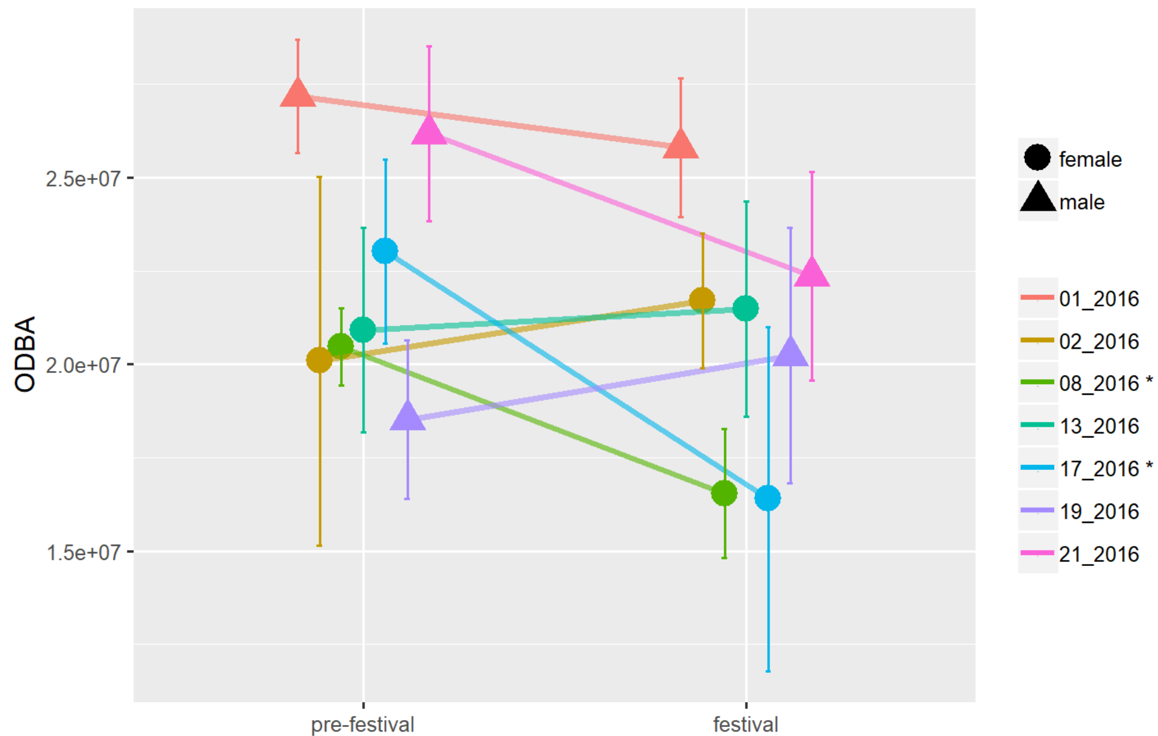


Figure 5.5: Mean values with confidence interval for the Overall Dynamic Body Acceleration (ODBA) of seven hedgehogs in the pre-festival and festival timeframe (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour.

5.3.7 Stress detection

During the festival phase, six out of eight hedgehogs decreased their DFC. For five out of eight hedgehogs these differences were significant, indicated by a Wilcoxon rank sum (08_2016, 09_2016, 13_2016, 19_2016, 21_2016) (Figure 5.6).

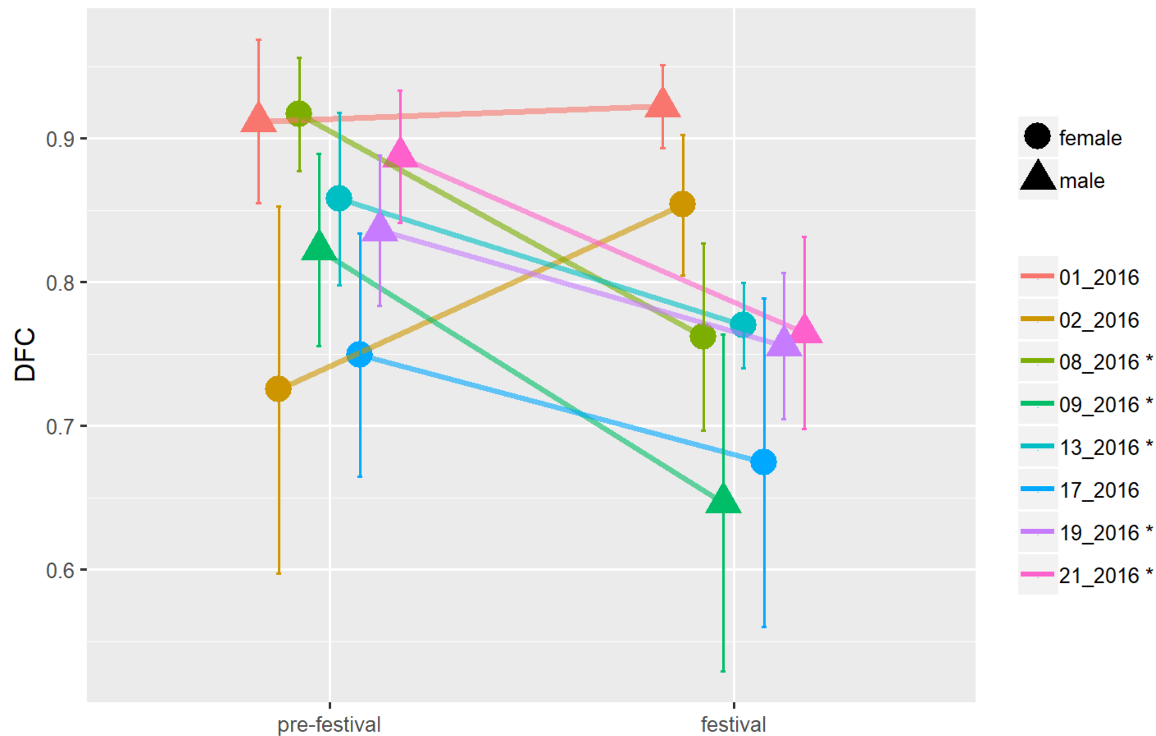


Figure 5.6: Mean values with confidence interval for the Degrees of Functional Coupling (DFC) of eight hedgehogs in the undisturbed and disturbed timeframe (significant differences are marked by *, Wilcoxon test (Table S1)). Female hedgehogs are represented as circles, male hedgehogs as triangles. Each individual is represented by a unique colour.

5.4 Discussion

We showed that all hedgehogs change their spatial-temporal behaviour in at least one of our study parameters during a large disturbance event. Following our predictions the area used nightly (KDE50) and DFC decreased during the festival. We were able to identify individual changes in the behavioural budget due to the festival. We discuss the different parameters below.

5.4.1 Spatial behaviour

We demonstrated a decrease in the area used nightly of urban hedgehogs during the festival. Our results are consistent with the recent meta-analysis of [36] reporting a widespread decrease in the mobility of mammals living in highly disturbed environments. They suggested that animals living in built-up landscapes were confined to smaller ranges due to limited movement capacity. During our study, movement limitations set by the park boundaries for hedgehogs were unchanged, thus a decrease in the area used nightly during the festival phase seems to be an effect of avoidance of disturbance caused by the festival.

In general, hedgehogs regularly change their nests [13, 37]. However, this was the first time a survival analysis was performed on the nesting behaviour so there is no relatable data for the pre-festival phase. Building additional nests requires a time investment to find appropriate nesting

sites and gather material, which could otherwise be used for foraging. Nevertheless, males changed their nests more often in the festival period. Because nesting behaviour is an important part of hedgehog behaviour, a change could be considered as a drastic reaction to the festival. Nests used by the females were used longer which could be explained by the fact that some of them gave birth and thus were bound to their nests. Changing nests with offspring is even more energetically costly than changing by themselves. Highly disturbed mothers might eat their offspring [13] and then change their nest.

5.4.2 Behaviour analysis

In contrast to spatial behaviour, hedgehogs did not change their individual behaviours in the festival phase relative to the pre-festival phase in a uniform way. Due to the nocturnal behaviour of hedgehogs, any behavioural observation would usually require an observer to be close to the animal to classify behaviour. This, however, could already lead to an influence on hedgehog behaviour [38]. This study is the first study to remotely record hedgehog behaviour, and there are no references as to how hedgehogs behave in the absence of a human observer.

Four hedgehogs reduced the amount of immobile behaviour while the three others showed no change (Table 5.3). The interpretation here is difficult because the situations in which a hedgehog may become immobile can be quite different. Hedgehogs will be classified as immobile if they stop walking during foraging or when they are in their nests sleeping during the daytime. The reduction in immobile behaviour here could mean a different sleeping posture that is more similar to balling up and therefore treated as such by the SVM. All four individuals that show reduced immobile behaviour also showed increased balling up behaviour. In addition to these four individuals, two other hedgehogs showed an increase in balling up during the festival. Nevertheless balling up is a defensive behaviour which is favoured by hedgehogs over moving away from a threat [13]. In a case where construction workers and the music event are perceived as a threat by the hedgehogs an increase in balling up would be expected and was observed in most of the study hedgehogs.

Interestingly two females (02_2016 and 13_2016) reduced their balling up behaviour during the festival phase. Direct observation of those two confirmed that both had offspring during the study. Judging from the developmental state of the offspring 02_2016 is estimated to have given birth in the last week before the festival (22nd of August until the 28th of August) and 13_2016 in the first week of the festival (29th of August until the 4th of September). Having offspring in the nest could prevent the mothers from balling up for either the simple reason that the nest does not offer enough space or the fact that balling up would prevent the offspring from

reaching the teats. The change in balling up behaviour was more drastic in 02_2016. Considering the estimated date of birth, the offspring of 02_2016 should have been bigger than the offspring of 13_2016 during the whole festival. This would support the hypothesis of the lack of space in the nest as a reason for reduced balling up. It has to be noted that there are different degrees of balling up. For this study, balling up was defined as a complete ball with no visible head. There are however defensive positions a hedgehog can assume were the balling is only minimal [13]. This could lead to a misclassification of a defensive behaviour as a non-defensive immobile behaviour.

A number of mammals have been shown to reduce their movement when in the presence of a predator [39]. In this context, a reduction in locomotion would have been expected. However, the effect of the festival on locomotive behaviour is mixed between the individuals. There were no significant differences among males, suggesting that the festival had no influence on their locomotion. All females, however, showed a significant change in locomotion. Individuals 08_2016 and 17_2016 showed reduced locomotion while 02_2016 and 13_2016 showed an increase. As the latter two were in lactation they should have experienced a higher energy demand than the females with no offspring [40]. Therefore they were forced to increase their foraging effort regardless of the festival while the other two females could avoid taking longer trips.

5.4.3 Daily activity pattern

Ordiz et al. [41] showed that changes in daily activity patterns are useful as a proxy of anthropogenic influences on wildlife. While, Gaynor et. al [42] showed, irrespective of taxa, habitat or location, mammals were more nocturnal in their daily activity patterns in response to human disturbance. For hedgehogs as strict nocturnal animals, increased nocturnal behaviour is difficult. Indeed, during the festival phase, six out of eight hedgehogs shifted their activity even more into the night shown by a decrease in DIs. The one animal (17_2016) which highly increased DI during the festival had its nest below a food stand near the stage and was observed feeding on food scraps during the day, which is both an unusual behaviour and food source for hedgehogs. However, only considering the DI did not effectively show how much hedgehog activity pattern changed during the festival. TSdusk showed that the hedgehogs started their nocturnal activity later during the festival than before the festival. Similarly, Shirley et al. [43] showed that the Brinkburn Summer Music Festival had a significant effect on the timing of bat behaviour leaving their priory up to 47 min later on festival nights. Therefore, DI as a solitary

proxy for human disturbance is inadequate and should be supplemented by other parameters like TSdusk.

The extremely high confidence interval in the DI and TSdusk of female 02_2016 are due to a strongly disturbed activity pattern that most likely resulted from the parturition that was estimated for the last week of the pre-festival phase (see section 'behaviour analysis').

5.4.4 ODBA analysis

Overall it seems that the festival did not have any influence on the ODBA of the study hedgehogs. Only individuals 08_2016 and 17_2016 showed a significant decrease during the festival phase. Lower ODBA values could be the result of less movement in the nest during resting periods and also reduced foraging or general locomotion. It could be possible that those two individuals limited their movement in order to avoid contact with the construction works (compare with section 'Behaviour analysis' and [39]). It could also indicate that they moved less in their nests during resting periods to avoid being detected in the nest. Considering only the ODBA values, it is unclear why only these two showed changes in their ODBA profile and not the other five individuals that also inhabited the festival grounds during construction.

Using the ODBA as a proxy for energy expenditure seemed inappropriate in this study. Various circumstances could lead to ODBA values that do not properly reflect energy expenditure. Flexible tendons, different animal gaits and moving up or down slopes have an unknown influence on the ODBA to energy expenditure relationship [28]. In the case of hedgehogs, moving through an area with thick ground cover like ivy (*Hedera helix*), brambles (*Rubus spp.*) or a lot of dead wood is more taxing to hedgehogs than walking over an open field. The additional energy used will not be reflected in higher body acceleration and would lead to an underestimation of the energy expended. Additionally, hedgehogs have the ability to ball up. To hold this position the animal has to flex muscles which do not result in any body acceleration. This energy expenditure would be completely missed.

5.4.5 Stress detection

Out of eight hedgehogs, five showed a significant decrease in the DFC during the festival, which supports our hypothesis that DFC values were lower during the festival phase. High DFCs are often found in healthy animals or those which are strongly diurnal or nocturnal [44]. Low DFCs indicates that the animal is weakly synchronized with the environmental rhythm, which can be an indicator of stressors or disease, but also by parturition [45]. We interpret the changes in the DFC as a sign of stress [8]. Individual 02_2016 showed an increase in the DFC, although this

was not significant. This individual showed a wide confidence interval in the pre-festival phase suggesting low DFC on some days during this phase. As discussed in the section 'behaviour analysis', this female was estimated to have given birth in the last week of the pre-festival phase. Parturition was shown to have a great impact on the activity pattern of mothers [45]. Therefore high fluctuations in the DFC should be expected in the time around parturition. Even though individual 13_2016 also gave birth during the study no pattern similar to 02_2016 was recorded. In contrast, 17_2016 showed a wide confidence interval but no offspring were observed. Close monitoring throughout the study, however, led to the assumption that 17_2016 suffered from a miscarriage. Individual 01_2016 is the only hedgehog that shows almost no change in mean DFC values. Through our close spatial monitoring, we observed that this hedgehog left the study area and thus the festival grounds.

5.5 Conclusions

Even though urban hedgehogs are expected to show a greater tolerance towards anthropogenic activities compared to their rural conspecifics [12], we showed that the music festival had an impact on the behaviour of all study hedgehogs. However, there was no general pattern in the way hedgehogs reacted to the disturbance. Employing different strategies in the same environment was found to have an influence on the fitness of great tits [46].

Our study provides evidence of the strong behavioural plasticity of urban hedgehogs. Behavioural plasticity plays a key role in species adaptation to rapid environmental changes (like urbanization) caused by anthropogenic activities [6] and is also likely to be crucial in the context of coping strategies to human activities. Despite the high plasticity and higher abundance in urban than rural areas, numbers of hedgehogs have decreased across Europe [47–52]. Future management of hedgehogs in cities should therefore include spatial and temporal protection areas during human disturbances, like festival or park management measures.

We used seven different parameters to measure hedgehog behaviour (one using GPS, one using VHF and five using acceleration). We were able to map the behavioural reactions of eight hedgehogs in response to a festival event. The planning of the festival employed wildlife protection measures in building fences and closing the area during the night on the two days music was played. These measures seemed insufficient as indicated by the measurable hedgehog responses. We expect our findings to exemplify responses to disturbance in urban areas. Changes to the environment often happen on a large scale and appear very sudden. What remains unclear are the potential long-lasting effects of the festival event. Such effects could only be captured by long term monitoring. The presence of trucks and visitors during the festival might have

influenced soil density and the soil fauna which hedgehogs depend on as a food source. Soil compaction and its effect on hedgehogs have, to our knowledge, not yet been addressed. In a study in the Regent's Park in London [13] hedgehogs were found to avoid the sports area. Here it was also suggested that the compaction due to sport activities had an impact on the prey species of the hedgehog, and it has been shown that soil compaction can influence the abundance of earthworms [53] which hedgehogs rely on as a food source. As soil compaction is not quick to reverse, possible long term effects should be considered in future studies.

Habituation to humans may indeed appear when animals are repeatedly exposed to benign interactions with human activities, although differences exist in the degree to which a species, or an individual, tolerates humans [54, 55]. We found a high individuality in our study and therefore strongly recommend that future studies and management plans consider the potential influence of the individuality of solitary species and provide retreat areas [56].

5.6 Acknowledgements

We would like to thank Feona Oltmann, Doreen Müller, Annika Krüger, Judith Niedersen, Juline Cibis and Catrin Zordick for assistance during field work. We thank the Lollapalooza organising committee for allowing us to access the festival ground during the festival phase. We also thank Dr. Lysanne Sniders, Dr. Tanja Straka and Dr. Liam Bailey for their improvement of the manuscript.

Funding

This work was partly funded by the German Federal Ministry of Education and Research BMBF within the Collaborative Project 'Bridging in Biodiversity Science - BIBS' (grant number 01LC1501A-H) and the Leibniz Institute for Zoo and Wildlife Research (IZW). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (IZW permit 2016-02-01) and German federal law (permission number Reg0115/15 and G0104/14).

Conflicts of Interest: 'The authors declare no conflict of interest.'

5.7 References

1. Munguía, M.; Trejo, I.; González-Salazar, C.; Pérez-Maqueo, O. Human impact gradient on mammalian biodiversity. *Glob. Ecol. Conserv.* **2016**, *6*, 79–92.
2. Grimm, N.B.; Faeth, S.H.; Golubiewski, N.E.; Redman, C.L.; Wu, J.; Bai, X.; Briggs, J.M. Global Change and the Ecology of Cities. *Science* **2008**, *319*, 756–760.
3. Seto, K.C.; Guneralp, B.; Hutyra, L.R. Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. *Proc. Natl. Acad. Sci.* **2012**, *109*, 16083–16088.
4. Lowry, H.; Lill, A.; Wong, B.B.M. Behavioural responses of wildlife to urban environments: Behavioural responses to urban environments. *Biol. Rev.* **2013**, *88*, 537–549.
5. Fischer, J.D.; Schneider, S.C.; Ahlers, A.A.; Miller, J.R. Categorizing wildlife responses to urbanization and conservation implications of terminology: Terminology and urban conservation. *Conserv. Biol.* **2015**, *29*, 1246–1248.
6. Sih, A.; Ferrari, M.C.O.; Harris, D.J. Evolution and behavioural responses to human-induced rapid environmental change. *Evol. Appl.* **2011**, *4*, 367–387.
7. Gattermann, R.; Johnston, R.E.; Yigit, N.; Fritzsche, P.; Larimer, S.; Özkurt, S.; Neumann, K.; Song, Z.; Colak, E.; Johnston, J.; et al. Golden hamsters are nocturnal in captivity but diurnal in nature. *Biol. Lett.* **2008**, *4*, 253–255.
8. Berger, A.; Scheibe, K.-M.; Michaelis, S.; Streich, W.J. Evaluation of living conditions of free-ranging animals by automated chronobiological analysis of behavior. *Behav. Res. Methods Instrum. Comput.* **2003**, *35*, 458–466.
9. Berger, A. Activity patterns, chronobiology and the assessment of stress and welfare in zoo and wild animals: STRESS ASSESSMENT BY APPLIED CHRONOBIOLOGY. *Int. Zoo Yearb.* **2011**, *45*, 80–90.
10. Williams, B.M.; Baker, P.J.; Thomas, E.; Wilson, G.; Judge, J.; Yarnell, R.W. Reduced occupancy of hedgehogs (*Erinaceus europaeus*) in rural England and Wales: The influence of habitat and an asymmetric intra-guild predator. *Sci. Rep.* **2018**, *8*.
11. Poel, J.L. van de; Dekker, J.; Langevelde, F. van Dutch hedgehogs *Erinaceus europaeus* are nowadays mainly found in urban areas, possibly due to the negative Effects of badgers *Meles meles*. *Wildl. Biol.* **2015**, *21*, 51–55.
12. Hubert, P.; Julliard, R.; Biagianti, S.; Poulle, M.-L. Ecological factors driving the higher hedgehog (*Erinaceus europeaus*) density in an urban area compared to the adjacent rural area. *Landsc. Urban Plan.* **2011**, *103*, 34–43.
13. Morris, P. *Hedgehog*; 2018; ISBN 978-0-00-823573-4.
14. Stache, A.; Heller, E.; Hothorn, T.; Heurich, M. Activity patterns of European roe deer (*Capreolus capreolus*) are strongly influenced by individual behaviour. *Folia Zool.* **2013**, *62*, 67–75.
15. Lesmerises, R.; St-Laurent, M.-H. Not accounting for interindividual variability can mask habitat selection patterns: a case study on black bears. *Oecologia* **2017**, *185*, 415–425.
16. Mori, E.; Menchetti, M.; Bertolino, S.; Mazza, G.; Ancillotto, L. Reappraisal of an old cheap method for marking the European hedgehog. *Mammal Res.* **2015**, *60*, 189–193.
17. Reeve, N.; Bowen, C.; Gurnell, J. An improved identification marking method for hedgehogs.
18. Barthel, L.M.F.; Hofer, H.; Berger, A. An easy, flexible solution to attach devices to hedgehogs (*Erinaceus europaeus*) enables long-term high-resolution studies. *Ecol. Evol.* **2019**, *9*, 672–679.
19. Sikes, R.S.; Gannon, W.L. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *J. Mammal.* **2011**, *92*, 235–253.

20. Calenge, C. The package 'adehabitat' for the R software: A tool for the analysis of space and habitat use by animals. *Ecol. Model.* **2006**, *197*, 516–519.
21. R Core Team *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018;
22. RStudio Team *RStudio: Integrated Development Environment for R*; RStudio, Inc.: Boston, MA, 2016;
23. Terry M. Therneau; Patricia M. Grambsch *Modeling Survival Data: Extending the Cox Model*; Springer: New York, 2000; ISBN 0-387-98784-3.
24. Cibis, J. Automatic behaviour classification from three-axial acceleration data for the European hedgehog (*Erinaceus europaeus* L., 1758). Master Thesis, Humbold-Universität zu Berlin: Berlin, 2017.
25. Rast, W. accelerateR: Process acceleration data; 2019; https://github.com/wanjarast/accelerateR
26. Nathan, R.; Spiegel, O.; Fortmann-Roe, S.; Harel, R.; Wikelski, M.; Getz, W.M. Using tri-axial acceleration data to identify behavioral modes of free-ranging animals: general concepts and tools illustrated for griffon vultures. *J. Exp. Biol.* **2012**, *215*, 986–996.
27. Collins, P.M.; Green, J.A.; Warwick-Evans, V.; Dodd, S.; Shaw, P.J.A.; Arnould, J.P.Y.; Halsey, L.G. Interpreting behaviors from accelerometry: a method combining simplicity and objectivity. *Ecol. Evol.* **2015**, *5*, 4642–4654.
28. Wilson, R.P.; White, C.R.; Quintana, F.; Halsey, L.G.; Liebsch, N.; Martin, G.R.; Butler, P.J. Moving towards acceleration for estimates of activity-specific metabolic rate in free-living animals: the case of the cormorant: Activity-specific metabolic rate in free-living animals. *J. Anim. Ecol.* **2006**, *75*, 1081–1090.
29. Cortes, C.; Vapnik, V. Support-vector networks. *Mach. Learn.* **1995**, *20*, 273–297.
30. Martiskainen, P.; Järvinen, M.; Skön, J.-P.; Tiirikainen, J.; Kolehmainen, M.; Mononen, J. Cow behaviour pattern recognition using a three-dimensional accelerometer and support vector machines. *Appl. Anim. Behav. Sci.* **2009**, *119*, 32–38.
31. Bidder, O.R.; Campbell, H.A.; Gómez-Laich, A.; Urgé, P.; Walker, J.; Cai, Y.; Gao, L.; Quintana, F.; Wilson, R.P. Love Thy Neighbour: Automatic Animal Behavioural Classification of Acceleration Data Using the K-Nearest Neighbour Algorithm. *PLoS ONE* **2014**, *9*, e88609.
32. Meyer, D.; Dimitriadou, E.; Hornik, K.; Weingessel, A.; Leisch, F. e1071: misc functions of the department of statistics, probability theory group (Formerly: E1071), TU Wien. R package version 3.5.1; 2017;
33. Hoogenboom, I.; Daan, S.; Dallinga, J.H.; Schoenmakers, M. Seasonal change in the daily timing of behaviour of the common vole, Microtus arvalis. *Oecologia* **1984**, *61*, 18–31.
34. Halsey, L.G.; Shepard, E.L.C.; Quintana, F.; Gomez Laich, A.; Green, J.A.; Wilson, R.P. The relationship between oxygen consumption and body acceleration in a range of species. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **2009**, *152*, 197–202.
35. Qasem, L.; Cardew, A.; Wilson, A.; Griffiths, I.; Halsey, L.G.; Shepard, E.L.C.; Gleiss, A.C.; Wilson, R. Tri-Axial Dynamic Acceleration as a Proxy for Animal Energy Expenditure; Should We Be Summing Values or Calculating the Vector? *PLoS ONE* **2012**, *7*, e31187.
36. Tucker, M.A.; Böhning-Gaese, K.; Fagan, W.F.; Fryxell, J.M.; Moorter, B.V.; Alberts, S.C.; Ali, A.H.; Allen, A.M.; Attias, N.; Avgar, T.; et al. Moving in the Anthropocene: Global reductions in terrestrial mammalian movements. *Science* **2018**, *359*, 466–469.
37. Reeve, N.J.; Morris, P.A. Construction and use of summer nests by the hedgehog (*Erinaceus europaeus*). *Mammalia* **1985**, *49*, 187–194.
38. Schneirla, T.C. The relationship between observation and experimentation in the field study of behavior. *Ann. N. Y. Acad. Sci.* **1950**, *51*, 1022–1044.

39. Lima, S.L. Stress and Decision Making under the Risk of Predation: Recent Developments from Behavioral, Reproductive, and Ecological Perspectives. In *Advances in the Study of Behavior*; Elsevier, 1998; Vol. 27, pp. 215–290 ISBN 978-0-12-004527-3.
40. Speakman, J.R. The physiological costs of reproduction in small mammals. *Philos. Trans. R. Soc. B Biol. Sci.* **2008**, *363*, 375–398.
41. Ordiz, A.; Kindberg, J.; Sæbø, S.; Swenson, J.E.; Støen, O.-G. Brown bear circadian behavior reveals human environmental encroachment. *Biol. Conserv.* **2014**, *173*, 1–9.
42. Gaynor, K.M.; Hojnowski, C.E.; Carter, N.H.; Brashares, J.S. The influence of human disturbance on wildlife nocturnality. *Science* **2018**, *360*, 1232–1235.
43. Shirley, M.D.F.; Armitage, V.L.; Barden, T.L.; Gough, M.; Lurz, P.W.W.; Oatway, D.E.; South, A.B.; Rushton, S.P. Assessing the impact of a music festival on the emergence behaviour of a breeding colony of Daubenton's bats (Myotis daubentonii). *J. Zool.* **2001**, *254*, 367–373.
44. Krone, O.; Berger, A.; Schulte, R. Recording movement and activity pattern of a White-tailed Sea Eagle (Haliaeetus albicilla) by a GPS datalogger. *J. Ornithol.* **2009**, *150*, 273–280.
45. Langbein, J.; Scheibe, K.-M.; Eichhorn, K. Investigations on periparturient behaviour in free-ranging mouflon sheep (Ovis orientalis musimon). *J. Zool.* **1998**, *244*, 553–561.
46. Both, C.; Dingemanse, N.J.; Drent, P.J.; Tinbergen, J.M. Pairs of extreme avian personalities have highest reproductive success. *J. Anim. Ecol.* **2005**, *74*, 667–674.
47. Hof, A.R. A study of the current status of the hedgehog (*Erinaceus europaeus*), and its decline in Great Britain since 1960. PhD Thesis, Royal Holloway, University of London: London, 2009.
48. Johnson, H.; Reeve, N.J.; Baker, P.; Yarnell, R.; Allen, S.; Petrovan, S. Conservation strategy for West-European Hedgehog (*Erinaceus europaeus*) in the United Kingdom (2015–2025). *Lond. UK People Trust Endangerd Species Br. Hedgehog Preserv. Soc.* **2015**.
49. Müller, F. Langzeit-Monitoring der Straßenverkerhsopfer beim Igel (*Erinaceus europaeus* L.) zur Indikation von Populationsdichteveränderungen entlang zweier Teststrecken im Landkreis Fulda. *Beitr. Zur Naturkunde Osthess.* **2018**, *54*, 21–26.
50. Pettett, C.E.; Johnson, P.J.; Moorhouse, T.P.; Macdonald, D.W. National predictors of hedgehog *Erinaceus europaeus* distribution and decline in Britain. *Mammal Rev.* **2018**, *48*, 1–6.
51. Roos, S.; Johnston, A.; Noble, D. UK hedgehog datasets and their potential for long-term monitoring. *BTO Res. Rep.* **2012**, *598*, 1–63.
52. Wilson, E.; Wembridge, D. The State of Britain's Hedgehogs 2018. *People's Trust Endanger. Species Br. Hedgehog Preserv. Soc.* **2018**. https://www. hedgehogstreet. org/wp-content/uploads/2018/02/SoBH-2018_final. pdf
53. Bottinelli, N.; Capowiez, Y.; Ranger, J. Slow recovery of earthworm populations after heavy traffic in two forest soils in northern France. *Appl. Soil Ecol.* **2014**, *73*, 130–133.
54. Geffroy, B.; Samia, D.S.M.; Bessa, E.; Blumstein, D.T. How Nature-Based Tourism Might Increase Prey Vulnerability to Predators. *Trends Ecol. Evol.* **2015**, *30*, 755–765.
55. Samia, D.S.M.; Nakagawa, S.; Nomura, F.; Rangel, T.F.; Blumstein, D.T. Increased tolerance to humans among disturbed wildlife. *Nat. Commun.* **2015**, *6*.
56. Réale, D.; Reader, S.M.; Sol, D.; McDougall, P.T.; Dingemanse, N.J. Integrating animal temperament within ecology and evolution. *Biol. Rev.* **2007**, *82*, 291–318.

General discussion

A sustainable population, which is resistant and resilient to environmental changes, is an essential base for species survival. The genetic diversity and behaviour of individuals are primary drivers of population sustainability. During this project, we investigated hedgehogs over the whole city of Berlin and beyond to reveal possible effects of the urban matrix on hedgehogs. With improvements in genetic and ecological methods, we have laid the foundation for future projects. Furthermore, we reveal complex population reactions as well as the individual coping strategies of an enigmatic small mammal to different stressors in the anthropogenic changed environment. Hedgehogs could serve as a model or even flagship species for small ground-dwelling species in urban systems for applied evidence-based conservation, by utilizing basic ecological understanding on population and individual levels.

Genetic of hedgehogs in urban systems

With the aide of stakeholders, we non-invasively collected saliva samples from individuals in and around Berlin to analyse genetic composition via microsatellite data. Surprisingly we found no signs of genetic clustering in the first citywide genetic study of this area (Berlin 875.94 km^2). We expected some sort of genetic structure in the population, based on the traits of the species and limitation of dispersal in highly fragmented habitat and previous results in a smaller urbanized area (Zurich <100 km^2). The lack of a general genetic differentiation across the entire city and some surrounding areas deserve some discussion, even though our study revealed some fine-grained population structure in some local parks. In the first approach using a 'cleaned' dataset with only unrelated samples, all samples were assigned to one cluster, while in a second approach with all available samples 'family-clans' in some areas parks were found. This could be the first sign of restricted gene flow in the population of hedgehogs. In combination with inbreeding and initial population size, this could increase the probability of local population extinction (Soul and Mills, 1998). However, the clustering approaches are sensitive to analyses with close relatives, like half and full siblings (Rodríguez-Ramilo and Wang, 2012), which are naturally spatial close to each other if they survive. In hedgehogs, a minimum viable population of 32 individuals on urban green patches with at least 90 ha was identified by a simulation model (Moorhouse, 2013). Therefore in theory, both parks investigated here, showing 'family-clan' structures, could be self-sustaining and disconnected to the surrounding population. This could explain how green spaces support hedgehog populations in urban areas but does not explain the general lack of structure over the whole city, considering the more conservative approach to our genetic analysis. It could

be possible that additional factors have enhanced genetic mixing. Considering the high numbers of hedgehogs in animal rehabilitation centres and their subsequent release into subjectively favourable habitats, we suggest that welfare activities could lead to the lack of structure, which we call the 'welfare disturbance hypothesis'. The welfare disturbance hypothesis includes the fact that rehabilitated hedgehogs are rarely released at their point of origin. Hedgehogs usually have limited opportunities for dispersal across the city, due to no clear dispersal phase in their ontogeny and their susceptibility to barriers. Therefore this human facilitated dispersal could lead to increased genetic diversity of the population. Thus, if this hypothesis is true, all remaining populations and potential structuring would be masked by the released hedgehogs. In order to study the effect of translocation after rehabilitation on genetic diversity, two things would have to be tested; first, we would need to study clear examples of hedgehogs that have been translocated and secondly whether or not they have successfully reproduced in the new environment, enhancing admixture and increasing genetic diversity.

Higher resolution in genetic methods helps to identify finer differences between individuals/subpopulations and increase the understanding of genetic dynamics within the population. Our improved methods and newly established genetic markers provide this higher resolution (see Chapter 2 / (ii)). By identifying 42 genetic markers for closely related hedgehog species instead of only 10 markers in one species, it has been possible to detect fine structural elements. Furthermore, now it is possible to detect potential hybridization between the two species. Interestingly in the process of identifying this marker, only in *E. roumanicus* we found a relationship between genetic structure and geographical distribution that was absent in *E. europaeus*, obscuring more interesting traits, which could be revealed by Europe wide collaboration. Using relatively easily accessible samples from former projects or museum samples, plus new non-invasive saliva samples from collaborations with veterinary practices, rehabilitation centres, and animal shelters, new projects should be possible in the nearer future. If in this process the effect described by the welfare disturbance hypotheses is confirmed, it could weaken the potential of hedgehogs as model species, to identify early signs of critical levels of fragmentation. However, 'urban conservation genetics' need model species from various taxa to give a more complete picture about the genetic variability of urban wildlife (Noël and Lapointe, 2010; Munshi-South and Nagy, 2014). In a study of urban mice, only 3 out of 14 populations showed consequences of genetic bottlenecks, while small networks of green spaces seemed to support self-sustaining populations (Munshi-South and Nagy, 2014). Our first question, whether fragmentation has affected the genetic structure of the hedgehog population in Berlin, is partly answered since there is no sign of spatial clustering. Understanding the

underlying mechanisms could lead to evidence-based population management instead of passively relying on the current genetic diversity and the unknown effects of possible animal movement by wildlife rehabilitators.

Movement and behaviour

Genetic diversity and mixture of subpopulations are strongly connected to movement and dispersal of animals. Thus, our new method to attach data loggers on hedgehogs enables long-term ecological studies to understand the movements of hedgehogs in and outside of cities (iii). Compared to previous methods, this novel method has not only improved welfare of studied individuals, it also decreased general costs and improved flexibility to study hedgehogs in a natural setting. It also enabled us, together with technological advancements to studying hedgehogs with higher spatial and temporal resolution over a longer period than previous studies were able to. We showed (iv/v) that urban-living hedgehogs in Berlin display behavioural flexibility, which may be a response that enables the population to be resistant and resilient towards (anthropogenic) disturbances in the city. We observed individuals avoiding anthropogenic stressors and behavioural flexibility under new circumstances. It is only because of the application of this improved method that we could show that our investigated population changed their behaviour differently in response to anthropogenic disturbances. These disturbances influenced the behaviour not only spatially but also temporally: hedgehogs adjusted their roaming behaviour, their activity and nesting behaviour.

Considering the limitation in the statistical analyses of comparing two parks in two different years (iv), we found different adjustments of behaviour to disturbance on the one hand and fragmentation on the other. More precisely, we found individual coping strategies in the hedgehog population during the open-air music festival (v). These individual strategies are an example of the behavioural plasticity that may enable animals to cope with stressors and lead to a higher resistance to disturbances for the population and ultimately the species (McDonnell and Hahs, 2015). We were able to show that the circadian rhythm of hedgehogs changed with respect to the various stressors of the music festival. This change itself can be a sign of stress. Acute stress and chronic stress can affect the population negatively (Hofer and East, 1998). This is found to promote an impaired body condition and to permanently disrupt homeostasis, which for example lead to reduced reproduction, lower survival rates and lower immune response (Dhabhar *et al.*, 1996; Wingfield, Jacobs and Hillgarth, 1997; Buchanan, 2000; Sapolsky, Romero and Munck, 2000; Wikelski and Cooke, 2006; Demas *et al.*, 2011).

Animals could be pre-adapted or become habituated to the general urban stressors, living under constant stress similar to natural habitats (e.g. predation), but the acute and/or consistently changing stressors that occur in urban environments could have more detrimental long-term consequences. Our results could be considered evidence that hedgehogs, in general, are pre-adapted to cope with environmental changes within an urbanized matrix; however, we are not able to clarify if hedgehogs are thriving in the cities in the long-term. The question remains if hedgehogs can be considered as a resident species or if they make the best out of an unfavourable situation and are simply surviving, but not thriving, in cities. It could be that the behaviour of urban hedgehogs is maladjusted, leading to suboptimal behaviour and eventual extinction in urban habitats. The feedback system between environmental changes and behaviour must be better understood to finally evaluate the biodiversity of cities (Figure 0.2).

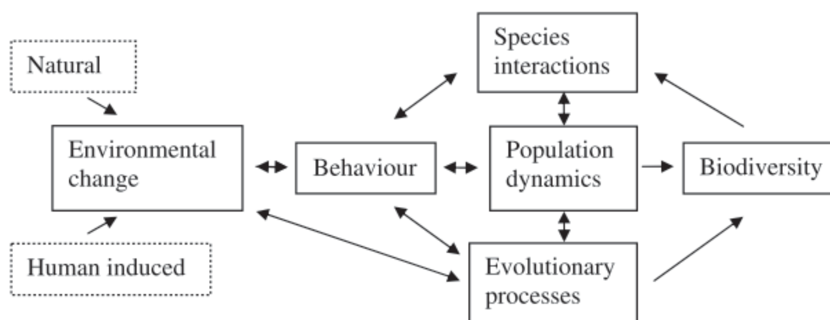


Figure 0.2: The feedback system between environment, behaviour and biodiversity. Changes in environmental conditions induce behavioural responses according to the reaction norm of the individual, which in turn affects species interactions, population dynamics, evolutionary processes and, ultimately, biodiversity. Changes in population dynamics, evolutionary processes and species interactions, in turn, affect behaviour, resulting in a complex network of feedback loops. From (Tuomainen and Candolin, 2011)

It has been previously described that animals react to disturbance and change their behaviour in response to environmental changes (McDonnell and Hahs, 2015). However, the effect of anthropogenic stressors is much larger and anthropogenic landscape changes occur at a faster rate than in most natural situations (Wong and Candolin, 2015). Additionally, in urban areas changes intensify over time, which could lead to a tipping point in the support of natural resources and species (Hastings *et al.*, 2018).

Past, present and future

Our results point out the restrictions of using hedgehogs as a model species for other small mammals and their genetic diversity. They do not seem to be affected in their genetic diversity or at least have enough factors supporting genetic mixing in the population, but further investigations should verify it. The hedgehogs still could serve as model and flagship species to investigate nature conservation, not only in the urban context. This species' close association to mosaic-like habitats, the predation on macro invertebrates and the need for dense and open vegetation could help to engage with the public and to convince hedgehog friendly park management and home gardening. These actions could support food webs and habitats for a variety of small mammals, birds, and other ecosystem services. We need to give nature space in this agricultural and urbanized environment (Beninde, Veith and Hochkirch, 2015; Villaseñor *et al.*, 2017). Particularly for hedgehogs, it would be preferable to create guidelines for hedgehog rescue centres and other animal rescue centres, to first keep track of the yearly numbers in this kind of facilities and to make evidence-based decisions of rehabilitation and informed relocation possible. We should not overlook other small mammal species in the cities and monitor them closely because it is possible that more species in the city can make the best out of their unfavourable situation. This could mean that, especially in huge pressure residential intensification, green spaces have to stay connected. Thus, the support of self-sustaining populations in urban areas could be possible and ensure a natural high genetic exchange. With respect to all species in cities, green parks should have 'natural' habitats where the park management is used to support natural processes. Leaving for example leaf litter can support the ecosystem at its foundation, with the maintenance of nutrient cyclings and invertebrate communities, which are supporting the food chain from ground-dwelling mammals to bats and birds. At the same time, these 'natural' areas create 'stepping-stones' for flora and fauna, enabling natural dispersal of species and connecting populations (Lundberg and Moberg, 2003). Even small gardens and crossing structures with native flora can connect and enable gene flow (Braaker, 2012; Braaker, Ghazoul, *et al.*, 2014; Braaker, Moretti, *et al.*, 2014; Soanes *et al.*, 2018). In order for the threatened and yet still numerous species in cities to be supported in future we need other knowledge and examples in urban planning to be integrated into infrastructural and landscape management of cities (Weisser and Hauck, 2015; Aronson *et al.*, 2017; Villaseñor *et al.*, 2017). Compact development minimizes the impacts of urban growth on native mammals (Villaseñor *et al.*, 2017).

Thus cities are able to create refuge habitats for species. It is possible that at some point in future populations from urbanized areas are needed to recolonize surrounding to restore natural

habitats. We need to understand the similarities and differences in the behaviour of species in both urban and rural context to protect both.

While we were able to show that research is necessary at both the individual and population level, without continued monitoring of individual movement and behaviours using specialized techniques such as ours, we will never be able to truly understand the greater intricacies of such a complex ecosystem as we have in urban centres. If anyone wants to preserve and understand the complexity of nature, an integrative and specialized approach is necessary. We now have the tools and the capacity to monitor animals at both fine and large spatial and temporal scales and this must be done to disentangle the intricate web of urban ecosystems.

Acknowledgments

First, I would like to thank all people who worked with me in the field: Feona, Annika, Judith, Juline, Catrin, Dominik, Hanna Lea, Phillip, Selina, Martin, Sara, Lu, Lenz, Andres, Jakopo, Katreen, Maria, Jennifer, Laurenz, Linda, Dana, Nadine, Lynn, Sharumathi, Saskia, Shanice and Henrik. Some of them did more for me than they know but all of us together made this project possible! I have to thank Doreen, Wanja, and Pierre for endless hours of analysing and discussing results. I would like to thank Anne Berger, Heribert Hofer and the IZW providing the project and workplace. Thank you to all my co-authors and colleagues at the IZW for cooperations, help and support where it was needed.

Furthermore, I have to thank all proof-readers that invest so much time in my still awful English, including Shannon, Tanja, Anne and Nigel, the stats group that taught me more than a lot of other stats courses over the years and the writing group initiated by Lysann, Shannon and Tanja that deserves a greater audience and helped to teach things every university should teach.

I have to thank my friends and colleagues, the old and new ones, that supported me during this project, pulled me out of some deep holes or just spent time listening, talking and dicussiong to and with me. These people are namely, to only name a few and if not already mentioned before: Stefanie, Sonia, Cecilia, Sophia, Caro, Benedict and Anne. I want to thank my parents Jutta Barthel and Bernhard Klein-Barthel. They made it possible that I could concentrate on my studies and helped and supported me in every decision I made. Finally, my appreciation goes to my girlfriend Indra, who not only supported me in my decision to work in a different city by putting a big burden on our relationship but also stand by me in this hard and instructive time.

Funding and animal permission

This work was partly funded by the German Federal Ministry of Education and Research BMBF within the Collaborative Project 'Bridging in Biodiversity Science - BIBS' (grant number 01LC1501A-H) and the Leibniz Institute for Zoo and Wildlife Research (IZW). All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (IZW permit 2016-02-01) and German federal law (permission number Reg0115/15 and G0104/14).

References from introduction and general discussion

Ansell, R. J., Baker, P. J. and Harris, S. (2001) 'The value of gardens for wildlife - lessons from mammals and herpetofauna', *British Wildlife*. BRITISH WILDLIFE PUBLISHING, 12, pp. 77–84.

Aronson, M. F. J., Lepczyk, C. A., Evans, K. L., Goddard, M. A., Lerman, S. B., MacIvor, J. S., Nilon, C. H. and Vargo, T. (2017) 'Biodiversity in the city: key challenges for urban green space management', *Frontiers in Ecology and the Environment*, 15(4), pp. 189–196. doi: 10.1002/fee.1480.

Baker, P. J. and Harris, S. (2007) 'Urban mammals: What does the future hold? An analysis of the factors affecting patterns of use of residential gardens in Great Britain', *Mammal Review*, 37(4), pp. 297–315. doi: 10.1111/j.1365-2907.2007.00102.x.

Bateman, P. W. and Fleming, P. A. (2012) 'Big city life: Carnivores in urban environments', *Journal of Zoology*, 287(1), pp. 1–23. doi: 10.1111/j.1469-7998.2011.00887.x.

Becher, S. a and Griffiths, R. (1997) 'Isolation and characterization of six polymorphic microsatellite loci in the European hedgehog *Erinaceus europaeus*.', *Molecular ecology*, 6(1), pp. 89–90. doi: 10.1046/j.1365-294X.1997.00159.x.

Belasen, A. M., Bletz, M. C., Leite, D. da S., Toledo, L. F. and James, T. Y. (2019) 'Long-Term Habitat Fragmentation Is Associated With Reduced MHC IIB Diversity and Increased Infections in Amphibian Hosts', *Frontiers in Ecology and Evolution*. Frontiers, 6, p. 236. doi: 10.3389/fevo.2018.00236.

Beninde, J., Veith, M. and Hochkirch, A. (2015) 'Biodiversity in cities needs space: A meta-analysis of factors determining intra-urban biodiversity variation', *Ecology Letters*, 18(6), pp. 581–592. doi: 10.1111/ele.12427.

Berger, A. (2011) 'Activity patterns, chronobiology and the assessment of stress and welfare in zoo and wild animals', *International Zoo Yearbook*, 45(1), pp. 80–90. doi: 10.1111/j.1748-1090.2010.00121.x.

Blair, R. B. (1996) 'Land Use and Avian Species Diversity Along an Urban Gradient', *Ecological Applications*. Ecological Society of America, 6(2), pp. 506–519. doi: 10.2307/2269387.

Bleicher, S. S. (2017) 'The landscape of fear conceptual framework: definition and review of current applications and misuses', *PeerJ*. PeerJ, Inc, 5, p. e3772. doi: 10.7717/peerj.3772.

Boitani, L. and Reggiani, G. (1984) 'Movements and activity patterns of Hedgehogs (*Erinaceus europaeus*) in Mediterranean coastal habitats', *Zeitschrift Säugetierkunde*, 49, pp. 193–206.

Bolfíková, B. Č., Eliášová, K., Loudová, M., Kryštufek, B., Lymberakis, P., Sándor, A. D. and Hulva, P. (2017) 'Glacial allopatry vs. postglacial parapatry and peripatry: the case of hedgehogs', *PeerJ*, 5, p. e3163. doi: 10.7717/peerj.3163.

Bolfíková, B. Č. and Hulva, P. (2012) 'Microevolution of sympatry: landscape genetics of hedgehogs Erinaceus europaeus and E. roumanicus in Central Europe', *Heredity*, 108, pp. 248–255. doi: 10.1038/hdy.2011.67.

Bolfíková, B., Konečný, A., Pfäffle, M., Skuballa, J. and Hulva, P. (2013) 'Population biology of

establishment in New Zealand hedgehogs inferred from genetic and historical data: conflict or compromise?', *Molecular Ecology*, 22(14), pp. 3709–3720. doi: 10.1111/mec.12331.

Braaker, S. (2012) *Habitat connectivity in an urban ecosystem*. ETH Zurich. doi: 10.3929/ethz-a-009787228.

Braaker, S., Ghazoul, J., Obrist, M. K. and Moretti, M. (2014) 'Habitat connectivity shapes urban arthropod communities: the key role of green roofs.', *Ecology*, 95(4), pp. 1010–1021. doi: 10.1890/13-0705.1.

Braaker, S., Moretti, M., Boesch, R., Ghazoul, J., Obrist, M. K. and Bontadina, F. (2014) 'Assessing habitat connectivity for ground-dwelling animals in an urban environment', *Ecological Applications*, 24(7), pp. 1583–1595. doi: 10.1890/13-1088.1.

Breck, S. W., Poessel, S. A., Mahoney, P. and Young, J. K. (2019) 'The intrepid urban coyote: a comparison of bold and exploratory behavior in coyotes from urban and rural environments', *Scientific Reports*. Springer US, 9(1), p. 2104. doi: 10.1038/s41598-019-38543-5.

Buchanan (2000) 'Stress and the evolution of condition-dependent signals.', *Trends in ecology & evolution*, 15(4), pp. 156–160. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10717685 (Accessed: 19 March 2019).

Burton, I. (1990) 'Factors in Urban Stress', *The Journal of Sociology & Social Welfare*, 17(1), p. 79. Available at: https://scholarworks.wmich.edu/jssw/vol17/iss1/5 (Accessed: 13 March 2019).

Burton, M. (1969) *The hedgehog*. London: Deutsch.

Demas, G. E., Zysling, D. A., Beechler, B. R., Muehlenbein, M. P. and French, S. S. (2011) 'Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts', *Journal of Animal Ecology*, 80(4), pp. 710–730. doi: 10.1111/j.1365-2656.2011.01813.x.

Dhabhar, F. S., Miller, A. H., McEwen, B. S. and Spencer, R. L. (1996) 'Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones.', *Journal of immunology (Baltimore, Md. : 1950)*, 157(4), pp. 1638–44. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8759750 (Accessed: 19 March 2019).

Diamond, J. M. (1988) 'Urban extinction of birds', *Nature*. Nature Publishing Group, 333(6172), pp. 393–394. doi: 10.1038/333393a0.

Díaz, S., Fargione, J., Chapin, F. S. and Tilman, D. (2006) 'Biodiversity Loss Threatens Human Well-Being', *PLoS Biology*. Public Library of Science, 4(8), p. e277. doi: 10.1371/journal.pbio.0040277.

Dickman, C. R. and Doncaster, C. P. (1988) 'The ecology of small mammals in urban habitats. II. Demography and dispersal', *Journal of Animal Ecology*, 58(1), pp. 119–127. doi: 10.2307/4989.

Ditchkoff, S. S., Saalfeld, S. T. and Gibson, C. J. (2006) 'Animal behavior in urban ecosystems: Modifications due to human-induced stress', *Urban Ecosystems*. Kluwer Academic Publishers, 9(1), pp. 5–12. doi: 10.1007/s11252-006-3262-3.

Doncaster, C. P. and Dickman, C. R. (1987) 'The ecology of small mammals in urban habitats. I. Population in a patchy environment', *Journal of Animal Ecology*, 56(2), pp. 629–640.

Duncan, R. P., Clemants, S. E., Corlett, R. T., Hahs, A. K., McCarthy, M. A., McDonnell, M. J., Schwartz,

M. W., Thompson, K., Vesk, P. A. and Williams, N. S. G. (2011) 'Plant traits and extinction in urban areas: a meta-analysis of 11 cities', *Global Ecology and Biogeography*. John Wiley & Sons, Ltd (10.1111), 20(4), pp. 509–519. doi: 10.1111/j.1466-8238.2010.00633.x.

Ellstrand, N. C. and Elam, D. R. (1993) 'Population Genetic Consequences of Small Population Size: Implications for Plant Conservation', *Annual Review of Ecology and Systematics*. Annual Reviews 4139 El Camino Way, P.O. Box 10139, Palo Alto, CA 94303-0139, USA, 24(1), pp. 217–242. doi: 10.1146/annurev.es.24.110193.001245.

Fischer, J. D., Schneider, S. C., Ahlers, A. A. and Miller, J. R. (2015) 'Categorizing wildlife responses to urbanization and conservation implications of terminology', *Conservation Biology*. John Wiley & Sons, Ltd (10.1111), 29(4), pp. 1246–1248. doi: 10.1111/cobi.12451.

Gaertner, M., Wilson, J. R. U., Cadotte, M. W., MacIvor, J. S., Zenni, R. D. and Richardson, D. M. (2017) 'Non-native species in urban environments: patterns, processes, impacts and challenges', *Biological Invasions*. Springer International Publishing, 19(12), pp. 3461–3469. doi: 10.1007/s10530-017-1598-7.

Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X. and Briggs, J. M. (2008) 'Global Change and the Ecology of Cities', *Science*, 319(5864), pp. 756–760. doi: 10.1126/science.1150195.

Guneralp, B. and Seto, K. C. (2013) 'Futures of global urban expansion: uncertainties and implications for biodiversity conservation', *Environmental Research Letters*, 8(1), p. 10. doi: 10.1088/1748-9326/8/1/014025.

Haigh, A., Butler, F., O'Riordan, R. and Palme, R. (2017) 'Managed parks as a refuge for the threatened red squirrel (Sciurus vulgaris) in light of human disturbance', *Biological Conservation*. Elsevier, 211(July), pp. 29–36. doi: 10.1016/j.biocon.2017.05.008.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., Stenmans, W., Müller, A., Sumser, H., Hörren, T., Goulson, D. and De Kroon, H. (2017) 'More than 75 percent decline over 27 years in total flying insect biomass in protected areas', *PLoS ONE*, 12(10). doi: 10.1371/journal.pone.0185809.

Hastings, A., Abbott, K. C., Cuddington, K., Francis, T., Gellner, G., Lai, Y. C., Morozov, A., Petrovskii, S., Scranton, K. and Zeeman, M. Lou (2018) 'Transient phenomena in ecology', *Science*, 361(6406). doi: 10.1126/science.aat6412.

Herter, K. (1965) *Hedgehogs: a comprehensive study*. London, (UK): Phoenix House.

Hof, A. R. (2009) *A study of the current status of the hedgehog (Erinaceus europaeus), and its decline in Great Britain since 1960*. Royal Holloway, University of London.

Hof, A. R. and Bright, P. W. (2010) 'The value of agri-environment schemes for macro-invertebrate feeders: Hedgehogs on arable farms in Britain', *Animal Conservation*, 13(5), pp. 467–473. doi: 10.1111/j.1469-1795.2010.00359.x.

Hof, A. R. and Bright, P. W. (2012) 'Factors affecting hedgehog presence on farmland as assessed by a

questionnaire survey', *Acta Theriologica*, 57(1), pp. 79–88. doi: 10.1007/s13364-011-0044-y.

Hof, A. R. and Bright, P. W. (2016) 'Quantifying the long-term decline of the West European hedgehog in England by subsampling citizen-science datasets', *European Journal of Wildlife Research*. European Journal of Wildlife Research, 62(4), pp. 407–413. doi: 10.1007/s10344-016-1013-1.

Hofer, H. and East, M. L. (1998) 'Biological conservation and stress', *Advances in the Study of Behavior*, 21(1), pp. 405–525. doi: 10.1016/S0065-3454(08)60370-8.

Hubert, P., Julliard, R., Biagianti, S. and Poulle, M.-L. L. M. (2011) 'Ecological factors driving the higher hedgehog (*Erinaceus europaeus*) density in an urban area compared to the adjacent rural area', *Landscape and Urban Planning*. Elsevier B.V., 103(1), pp. 34–43. doi: 10.1016/j.landurbplan.2011.05.010.

Huijser, M. P. (2000) 'Life on the edge. Hedgehog traffic victims and mitigation strategies in an anthropogenic landscape.', p. 165.

Huijser, M. P. and Bergers, P. J. M. (2000) 'The effect of roads and traffic on hedgehog (*Erinaceus europaeus*) populations', *Biological Conservation*, 95(1), pp. 111–116. doi: 10.1016/S0006-3207(00)00006-9.

Iannucci, A., Baccetti, N., Giannini, F., Gotti, C. and Baratti, M. (2018) 'A genetic analysis of the European hedgehog (Erinaceus europaeus): an applicative case study to support its eradication from Pianosa Island (Tuscan Archipelago)', *Conservation Genetics*. Springer Netherlands, pp. 1–8. doi: 10.1007/s10592-018-1078-3.

Ives, C. D., Lentini, P. E., Threlfall, C. G., Ikin, K., Shanahan, D. F., Garrard, G. E., Bekessy, S. A., Fuller, R. A., Mumaw, L., Rayner, L., Rowe, R., Valentine, L. E. and Kendal, D. (2016) 'Cities are hotspots for threatened species', *Global Ecology and Biogeography*, 25(1), pp. 117–126. doi: 10.1111/geb.12404.

Jackson, D. B. (2007) 'Factors affecting the abundance of introduced hedgehogs (*Erinaceus europaeus*) to the Hebridean island of South Uist in the absence of natural predators and implications for nesting birds', *Journal of Zoology*, 271(2), pp. 210–217. doi: 10.1111/j.1469-7998.2006.00204.x.

Jackson, D. B. and Green, R. E. (2000) 'The importance of the introduced hedgehog (*Erinaceus europaeus*) as a predator of the eggs of waders (*Charadrii*) on machair in South Uist, Scotland', *Biological Conservation*, 93(3), pp. 333–348. doi: 10.1016/S0006-3207(99)00135-4.

Johnson, H. (2015) 'Three years of the Hedgehog Hibernation Survey', (12).

Johnson, H., Reeve, N. J., Baker, P., Yarnell, R., Allen, S. and Petrovan, S. (2015) *Conservation Strategy for West-European Hedgehog (Erinaceus europaeus) in the United Kingdom (2015-2025)*. London.

Johnson, M. T. J. and Munshi-South, J. (2017) 'Evolution of life in urban environments', *Science*, 8327(November). doi: 10.1126/science.aam8327.

Kabisch, N., Strohbach, M., Haase, D. and Kronenberg, J. (2016) 'Urban green space availability in European cities', *Ecological Indicators*. Elsevier, 70, pp. 586–596. doi: 10.1016/j.ecolind.2016.02.029.

Kerley, G. I. H., Kowalczyk, R. and Cromsigt, J. P. G. M. (2012) 'Conservation implications of the

refugee species concept and the European bison: King of the forest or refugee in a marginal habitat?', *Ecography*, 35(6), pp. 519–529. doi: 10.1111/j.1600-0587.2011.07146.x.

Kowarik, I. (2011) 'Novel urban ecosystems, biodiversity, and conservation', *Environmental Pollution*. Elsevier, 159(8–9), pp. 1974–1983. doi: 10.1016/J.ENVPOL.2011.02.022.

Kowarik, I. and von der Lippe, M. (2018) 'Plant population success across urban ecosystems: a framework to inform biodiversity conservation in cities', *Journal of Applied Ecology*, (November 2017), pp. 1–8. doi: 10.1111/1365-2664.13144.

Krange, M. (2015) *Change in the occurrence of the West European Hedgehog (Erinaceus europaeus) in western Sweden during 1950-2010*. Karlstad University. Available at: http://kau.diva-portal.org/smash/record.jsf?pid=diva2%3A822331&dswid=9119 (Accessed: 5 December 2017).

Kristiansson, H. (1984) *Ecology of a hedgehog Erinaceus europaeus population in southern Sweden*. University of Lund, Sweden.

Kuemmerle, T., Hickler, T., Olofsson, J., Schurgers, G. and Radeloff, V. C. (2012) 'Refugee species: Which historic baseline should inform conservation planning?', *Diversity and Distributions*, 18(12), pp. 1258–1261. doi: 10.1111/ddi.12013.

LaPoint, S., Balkenhol, N., Hale, J., Sadler, J. and van der Ree, R. (2015) 'Ecological Connectivity Research in Urban Areas', *Functional Ecology*, 29, pp. 868–878. doi: 10.1111/1365-2435.12489.

Li, H. (2018) 'Urban Heat Island and its Influencing Mechanism In the City of Berlin', (April).

von der Lippe, M. and Kowarik, I. (2006) 'Die Vorgärten und Anlagen sind die naturbelebte Ergänzung der Wohnung "', in E. de Jong, E. S. & B. S. (ed.) *Der Garten–ein Ort des Wandels–Perspektiven fur die Denkmalpflege*. Zürich: vdf Hochschulverlag AG an der ETH Zürich, pp. 101–115.

Lowry, H., Lill, A. and Wong, B. B. M. M. (2013) 'Behavioural responses of wildlife to urban environments', *Biological Reviews*. Blackwell Publishing Ltd, 88(3), pp. 537–549. doi: 10.1111/brv.12012.

Lundberg, J. and Moberg, F. (2003) 'Mobile link organisms and ecosystem functioning: Implications for ecosystem resilience and management', *Ecosystems*, 6(1), pp. 87–98. doi: 10.1007/s10021-002-0150-4.

Magle, S. B., Hunt, V. M., Vernon, M. and Crooks, K. R. (2012) 'Urban wildlife research: Past, present, and future', *Biological Conservation*. Elsevier Ltd, 155, pp. 23–32. doi: 10.1016/j.biocon.2012.06.018.

McDonald, R. I. (2016) 'Urban ecology for the urban century', *Ecosystem Health and Sustainability*. John Wiley & Sons, Ltd, 2(7), p. e01221. doi: 10.1002/ehs2.1221.

McDonald, R. I., Güneralp, B., Huang, C. W., Seto, K. C. and You, M. (2018) 'Conservation priorities to protect vertebrate endemics from global urban expansion', *Biological Conservation*. doi: 10.1016/j.biocon.2018.06.010.

McDonnell, M. J. and Hahs, A. K. (2015) 'Adaptation and Adaptedness of Organisms to Urban Environments', *Annual Review of Ecology, Evolution, and Systematics*. Annual Reviews, 46(1), pp. 261–280. doi: 10.1146/annurev-ecolsys-112414-054258.

McKinney, M. L. (2002) 'Urbanization, Biodiversity, and Conservation', *BioScience*. Oxford University

Press, 52(10), p. 883. doi: 10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2.

Mills, G. (2008) 'Luke Howard andThe Climate of London', *Weather*, 63(6), pp. 153–157. doi: 10.1002/wea.195.

Miranda, A. C., Schielzeth, H., Sonntag, T. and Partecke, J. (2013) 'Urbanization and its effects on personality traits: A result of microevolution or phenotypic plasticity?', *Global Change Biology*, 19(9), pp. 2634–2644. doi: 10.1111/gcb.12258.

Moorhouse, T. P. (2013) *Report 2013 Population viability analysis of hedgehogs in rural and urban habitats*, *Wildlife Conservation Research Unit (WildCRU)*. Available at: http://ir.obihiro.ac.jp/dspace/handle/10322/3933.

Mori, E., Menchetti, M., Bertolino, S., Mazza, G. and Ancillotto, L. (2015) 'Reappraisal of an old cheap method for marking the European hedgehog', *Mammal Research*. Springer Berlin Heidelberg, 60(2), pp. 189–193. doi: 10.1007/s13364-014-0210-0.

Morris, P. A. (1973) 'Winter Nests of the Hedgehog (*Erinaceus europaeus* L.)', *Oecologia*, 11, pp. 299–313.

Müller, F. (2018) 'Langzeit-Monitoring der Straßenverkerhsopfer beim Igel (*Erinaceus europaeus* L.) zur Indikation von Populationsdichteveränderungen entlang zweier Teststrecken im Landkreis Fulda', *Beiträge zur Naturkunde in Osthessen*, 54, pp. 21–26.

Munshi-South, J. and Nagy, C. (2014) 'Urban park characteristics, genetic variation, and historical demography of white-footed mouse (Peromyscus leucopus) populations in New York City', *PeerJ*, 2, p. e310. doi: 10.7717/peerj.310.

Noël, S. and Lapointe, F. J. (2010) 'Urban conservation genetics: Study of a terrestrial salamander in the city', *Biological Conservation*, 143(11), pp. 2823–2831. doi: 10.1016/j.biocon.2010.07.033.

Noël, S., Ouellet, M., Galois, P. and Lapointe, F. J. (2007) 'Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander', *Conservation Genetics*, 8(3), pp. 599–606. doi: 10.1007/s10592-006-9202-1.

Pettett, C. E. (2016) 'Factors Affecting Hedgehog Distribution and Habitat Selection in Rural Landscapes'.

Pettett, C. E., Johnson, P. J., Moorhouse, T. P. and Macdonald, D. W. (2017) 'National predictors of hedgehog *Erinaceus europaeus* distribution and decline in Britain', *Mammal Review*, pp. 1–6. doi: 10.1111/mam.12107.

Pettett, C. E., Moorhouse, T. P., Johnson, P. J. and Macdonald, D. W. (2017) 'Factors affecting hedgehog (Erinaceus europaeus) attraction to rural villages in arable landscapes', *European Journal of Wildlife Research*. European Journal of Wildlife Research, 63(3), p. 12. doi: 10.1007/s10344-017-1113-6.

Poel, J. L. Van De, Dekker, J., Langevelde, F. Van, Poel, J. L. Van De, Dekker, J. and Langevelde, F. Van (2015) 'Dutch hedgehogs *Erinaceus europaeus* are nowadays mainly found in urban areas, possibly due to the negative Effects of badgers Meles meles', *Wildlife Biology*, 21(1), pp. 51–55. doi: 10.2981/wlb.00072.

Rautio, A., Valtonen, A. and Kunnasranta, M. (2013) 'The effects of sex and season on home range in

European hedgehogs at the northern edge of the species range', *Annales Zoologici Fennici*, 50(1–2), pp. 107–123. doi: 10.5735/086.050.0110.

Reeve, N. J. (1981) *A Field study of the hedgehog (*Erinaceus europaeus*) with particular peference to movements and behavior*. University of London.

Reeve, N. J. (1994) *Hedgehogs*. T & A. D Poyser, London.

Reeve, N. J. and Morris, P. A. (1985) 'Construction and use of summer nests by the hedgehog Erinaceus europaeus', *Mammalia*, 49(2), pp. 187–194. Available at: http://www.degruyter.com/view/j/mamm.1985.49.issue-2/mamm.1985.49.2.187/mamm.1985.49.2.187.xml (Accessed: 2 March 2016).

Reichholf, J. and Esser, J. (1981) 'Daten zur Mortalität des Igels (Erinaceus europaeus), verursacht durch den Straßenverkehr', *Z*, 46, pp. 216–222.

Rodríguez-Ramilo, S. T. and Wang, J. (2012) 'The effect of close relatives on unsupervised Bayesian clustering algorithms in population genetic structure analysis', *Molecular Ecology Resources*. doi: 10.1111/j.1755-0998.2012.03156.x.

Roos, S., Johnston, A. and Noble, D. (2012) 'UK Hedgehog datasets and their potential for long-term monitoring.', *British Trust for Ornothology Research Report*, (598), pp. 9–59. Available at: https://www.bto.org/sites/default/files/u12/hedgehogscopingreportfinal05042012ptes.pdf.

Santini, L., González-Suárez, M., Russo, D., Gonzalez-Voyer, A., von Hardenberg, A. and Ancillotto, L. (2018) 'One strategy does not fit all: determinants of urban adaptation in mammals', *Ecology Letters*. Edited by U. Brose. John Wiley & Sons, Ltd (10.1111), pp. 1–12. doi: 10.1111/ele.13199.

Santucci, F., Emerson, B. C. and Hewitt, G. M. (1998) 'Mitochondrial DNA phylogeography of European hedgehogs', *Molecular Ecology*, 7, pp. 1163–1172.

Sapolsky, R. M., Romero, L. M. and Munck, A. U. (2000) 'How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions [1]', *Endocrine Reviews*, 21(1), pp. 55–89. doi: 10.1210/edrv.21.1.0389.

Scheibe, K. M. and Gromann, C. (2006) 'Application testing of a new three-dimensional acceleration measuring system with wireless data transfer for behavior analysis', *Behavior Research Methods*, 38(3), pp. 427–433.

Seddon, J. M., Santucci, F., Reeve, N. J., Hewitt, G. M., Science, B., Seddon, J. M., Santucci, F., Reeve, N. J. and Hewitt, G. M. (2001) 'DNA footprints of European hedgehogs, Erinaceus europaeus and E. concolor: Pleistocene refugia, postglacial expansion and colonization routes', *Molecular Ecology*, 10(9), pp. 2187–2198. doi: 10.1046/j.0962-1083.2001.01357.x.

Seto, K. C., Güneralp, B. and Hutyra, L. R. (2012) 'Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 109(40), pp. 16083–8. doi: 10.1073/pnas.1211658109.

Seto, K. C., Parnell, S. and Elmqvist, T. (2013) 'A Global Outlook on Urbanization', in *Urbanization,*

Biodiversity and Ecosystem Services: Challenges and Opportunities. Dordrecht: Springer Netherlands, pp. 1–12. doi: 10.1007/978-94-007-7088-1_1.

Soanes, K., Taylor, A. C., Sunnucks, P., Vesk, P. A., Cesarini, S. and van der Ree, R. (2018) 'Evaluating the success of wildlife crossing structures using genetic approaches and an experimental design: Lessons from a gliding mammal', *Journal of Applied Ecology*. Edited by Y. Wiersma. Wiley/Blackwell (10.1111), 55(1), pp. 129–138. doi: 10.1111/1365-2664.12966.

Sol, D., Lapiedra, O. and González-Lagos, C. (2013) 'Behavioural adjustments for a life in the city', *Animal Behaviour*. Elsevier Ltd, 85(5), pp. 1101–1112. doi: 10.1016/j.anbehav.2013.01.023.

Soul, M. E. and Mills, L. S. (1998) 'No Need to Isolate Genetics', *Science*, 282(5394), pp. 1658–1659. doi: 10.1126/science.282.5394.1658.

Stirnemann, I. A., Lentini, P. E., Stein, J. A., Worthy, T. H., Stojanovic, D., Stirnemann, I. A., Stojanovic, D., Worthy, T. H. and Stein, J. A. (2017) 'Using fossil records to inform reintroduction of the kakapo as a refugee species', *Biological Conservation*. Elsevier, 217(March 2017), pp. 157–165. doi: 10.1016/j.biocon.2017.10.027.

Sutherland, W. J., Freckleton, R. P., Godfray, H. C. J., Beissinger, S. R., Benton, T., Cameron, D. D., Carmel, Y., Coomes, D. A., Coulson, T., Emmerson, M. C., Hails, R. S., Hays, G. C., Hodgson, D. J., Hutchings, M. J., Johnson, D., Jones, J. P. G., Keeling, M. J., Kokko, H., Kunin, W. E., Lambin, X., Lewis, O. T., Malhi, Y., Mieszkowska, N., Milner-Gulland, E. J., Norris, K., Phillimore, A. B., Purves, D. W., Reid, J. M., Reuman, D. C., Thompson, K., Travis, J. M. J., Turnbull, L. A., Wardle, D. A. and Wiegand, T. (2013) 'Identification of 100 fundamental ecological questions', *Journal of Ecology*, 101(1), pp. 58–67. doi: 10.1111/1365-2745.12025.

The State of Britain's Hedgehogs 2015 (2015) *Ptes*.

Threlfall, C. G., Mata, L., Mackie, J. A., Hahs, A. K., Stork, N. E., Williams, N. S. G. and Livesley, S. J. (2017) 'Increasing biodiversity in urban green spaces through simple vegetation interventions', *Journal of Applied Ecology*. doi: 10.1111/1365-2664.12876.

Threlfall, C. G., Ossola, A., Hahs, A. K., Williams, N. S. G., Wilson, L. and Livesley, S. J. (2016) 'Variation in Vegetation Structure and Composition across Urban Green Space Types', *Frontiers in Ecology and Evolution*, 4(June), pp. 1–12. doi: 10.3389/fevo.2016.00066.

Tucker, M. A., Böhning-Gaese, K., Fagan, W. F., Fryxell, J. M., Van Moorter, B., Alberts, S. C., Ali, A. H., Allen, A. M., Attias, N., Avgar, T., Bartlam-Brooks, H., Bayarbaatar, B., Belant, J. L., Bertassoni, A., Beyer, D., Bidner, L., van Beest, F. M., Blake, S., Blaum, N., Bracis, C., Brown, D., de Bruyn, P. J. N., Cagnacci, F., Calabrese, J. M., Camilo-Alves, C., Chamaillé-Jammes, S., Chiaradia, A., Davidson, S. C., Dennis, T., DeStefano, S., Diefenbach, D., Douglas-Hamilton, I., Fennessy, J., Fichtel, C., Fiedler, W., Fischer, C., Fischhoff, I., Fleming, C. H., Ford, A. T., Fritz, S. A., Gehr, B., Goheen, J. R., Gurarie, E., Hebblewhite, M., Heurich, M., Hewison, A. J. M., Hof, C., Hurme, E., Isbell, L. A., Janssen, R., Jeltsch, F., Kaczensky, P., Kane, A., Kappeler, P. M., Kauffman, M., Kays, R., Kimuyu, D., Koch, F., Kranstauber, B., LaPoint, S., Leimgruber, P., Linnell, J. D. C., López-López, P.,

Markham, A. C., Mattisson, J., Medici, E. P., Mellone, U., Merrill, E., de Miranda Mourão, G., Morato, R. G., Morellet, N., Morrison, T. A., Díaz-Muñoz, S. L., Mysterud, A., Nandintsetseg, D., Nathan, R., Niamir, A., Odden, J., O'Hara, R. B., Oliveira-Santos, L. G. R., Olson, K. A., Patterson, B. D., Cunha de Paula, R., Pedrotti, L., Reineking, B., Rimmler, M., Rogers, T. L., Rolandsen, C. M., Rosenberry, C. S., Rubenstein, D. I., Safi, K., Saïd, S., Sapir, N., Sawyer, H., Schmidt, N. M., Selva, N., Sergiel, A., Shiilegdamba, E., Silva, J. P., Singh, N., Solberg, E. J., Spiegel, O., Strand, O., Sundaresan, S., Ullmann, W., Voigt, U., Wall, J., Wattles, D., Wikelski, M., Wilmers, C. C., Wilson, J. W., Wittemyer, G., Zięba, F., Zwijacz-Kozica, T. and Mueller, T. (2018) 'Moving in the Anthropocene: Global reductions in terrestrial mammalian movements', *Science*, 359(6374), pp. 466–469. doi: 10.1126/science.aam9712.

Tuomainen, U. and Candolin, U. (2011) 'Behavioural responses to human-induced environmental change', *Biological Reviews*, 86(3), pp. 640–657. doi: 10.1111/j.1469-185X.2010.00164.x.

UN (United Nations) (2018) *2018 revision of world urbanization prospects*. New York, NY, USA.

Villaseñor, N. R., Tulloch, A. I. T., Driscoll, D. A., Gibbons, P. and Lindenmayer, D. B. (2017) 'Compact development minimizes the impacts of urban growth on native mammals', *Journal of Applied Ecology*, 54(3), pp. 794–804. doi: 10.1111/1365-2664.12800.

Wauters, L. A., Hutchinson, Y., Parkin, D. T. and Dhondt, A. A. (1994) 'The effects of habitat fragmentation on demography and on the loss of genetic variation in the red squirrel.', *Proceedings. Biological sciences*. The Royal Society, 255(1343), pp. 107–11. doi: 10.1098/rspb.1994.0015.

Weisser, W. and Hauck, T. (2015) *Animal Aided Design*. Available at: https://www.tum.de/die-tum/aktuelles/pressemitteilungen/detail/article/32308/.

Wikelski, M. and Cooke, S. J. (2006) 'Conservation physiology', *Trends in Ecology & Evolution*, 21(1), pp. 38–46. doi: 10.1016/j.tree.2005.10.018.

Williams, B. M., Baker, P. J., Thomas, E., Wilson, G., Judge, J. and Yarnell, R. W. (2018) 'Reduced occupancy of hedgehogs (*Erinaceus europaeus*) in rural England and Wales: The influence of habitat and an asymmetric intra-guild predator', *Scientific Reports*, 8(1), p. 12156. doi: 10.1038/s41598-018-30130-4.

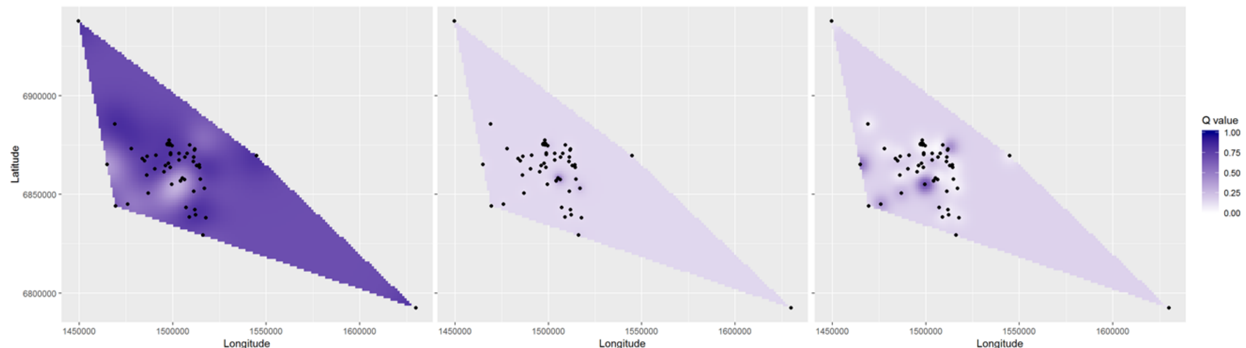
Williams, B., Mann, N., Neumann, J. L., Yarnell, R. W. and Baker, P. J. (2018) 'A prickly problem: developing a volunteer-friendly tool for monitoring populations of a terrestrial urban mammal, the West European hedgehog (*Erinaceus europaeus*)', *Urban Ecosystems*. Springer US, 21(6), pp. 1075–1086. doi: 10.1007/s11252-018-0795-1.

Wilson, E. and Wembridge, D. (2018) 'The State of Britain's Hedgehogs 2018', *Ptes*, pp. 1–24.

Wingfield, J. C., Jacobs, J. and Hillgarth, N. (1997) 'Ecological constraints and the evolution of hormone-behavior interrelationships.', *Annals of the New York Academy of Sciences*, 807, pp. 22–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9071342 (Accessed: 19 March 2019).

Wong, B. B. M. and Candolin, U. (2015) 'Behavioral responses to changing environments', *Behavioral Ecology*. Oxford University Press, 26(3), pp. 665–673. doi: 10.1093/beheco/aru183.

1 Supplementary information Chapter 1: Unexpected gene-flow in urban environments: the example of the European hedgehog



Supplementary Figure 1.1: Map of all sampling locations (N= 65), displaying the distribution of individual genotype Q-values (STRUCTURE analysis results), values in-between are interpolated. Left: data for cluster 1, center: data for cluster 2, right: data for cluster 3.

Supplementary Table 1.1: Origin of samples (N = 143)

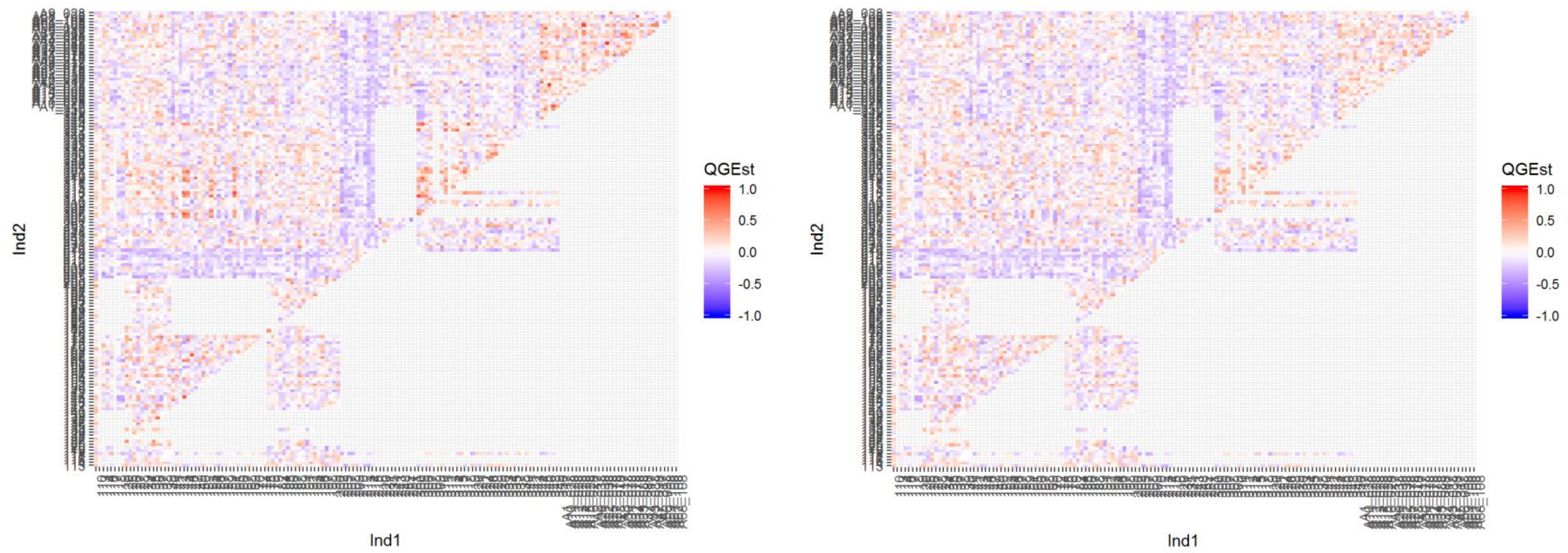
ID	Year	Month	Adress	lat_y	long_x	Source
110	2016	May	Trainierbahn Hoppegarten	52.4778846	13.6271494	IZW
127	2013	September	Rehwiese, Gerkrathstraße 2	52.42811	13.19973	IZW
128	2013	October	Moldaustr. 24, 10319 Berlin	52.49873	13.51299	Veterinary Practice
129	2013	October	Rohrwallallee 10, 12527 Berlin	52.39525	13.63471	Veterinary Practice
135	2013	October	Korkedamm 73, 12524 Berlin	52.4245	13.539	Veterinary Practice
136	2013	October	Moldaustr. 24, 10319 Berlin	52.49873	13.51299	Veterinary Practice
137	2013	September	Kablower Weg 89, 12526 Berlin	52.4034709	13.5853995	Veterinary Practice
138	2013	September	Kablower Weg 89, 12526 Berlin	52.4034709	13.5853995	Veterinary Practice
139	2013	September	Falkenberger Krugwiesen, 13057 Berlin	52.5641643	13.5338813	IZW
140	2013	September	Falkenberger Krugwiesen, 13057 Berlin	52.5641643	13.5338813	IZW
184	2016	July	Freischuetzstr., 13129 Berlin	52.6023802	13.45136	Veterinary Practice
185	2016	July	Gutenfelsstr. 14, 13129 Berlin	52.6005441	13.4493462	Veterinary Practice
186	2016	August	Gutenfelsstr. 14, 13129 Berlin	52.6005441	13.4493462	Veterinary Practice
187	2016	August	Gutenfelsstr. 14, 13129 Berlin	52.6005441	13.4493462	Veterinary Practice
188	2016	August	Schwarzelfenweg 19, 13088 Berlin	52.5714	13.46435	Veterinary Practice
189	2016	August	Strasse 7, 13129 Berlin	52.5983843	13.4486779	Veterinary Practice

191	2016	September	Strasse 26 Nr. 30, 13129 Berlin	52.595897	13.4727698	Veterinary Practice
192	2016	September	Urbacher Str., 13129 Berlin	52.6016518	13.4608262	Veterinary Practice
193	2016	October	Jungbornstr., 13129 Berlin	52.598014	13.4593083	Veterinary Practice
125	2013	September	Riesserseestr. 10, 12527 Berlin	52.4185357	13.5818458	Veterinary Practice
126	2013	September	Moldaustr. 30, 10319 Berlin	52.49834	13.51277	Veterinary Practice
134	2013	August	Volkspark Prenzlauerberg, Berlin	52.53564	13.4634372	IZW
176	2016	October	Eisenhuettenstadt	52.1436615	14.6419022	Hedgehog Station
179	2016	October	Eisenhuettenstadt	52.1436615	14.6419022	Hedgehog Station
182	2016	October	Zehlendorf, Berlin	52.4339586	13.2589089	Hedgehog Station
194	2016	October	Schwarzwaldstr./Ilsenstr., 13129 Berlin	52.598586	13.453231	Veterinary Practice
196	2016	October	Schwarzwaldstr., 13129 Berlin	52.598586	13.453231	Veterinary Practice
197	2016	November	Krontalerstr., 13125 Berlin	52.6112819	13.4577434	Veterinary Practice
198	2016	October	Gutenfelsstr. 14, 13129 Berlin	52.6005441	13.4493462	Veterinary Practice
199	2016	October	Hellersdorf,Berlin	52.536107	13.6049726	Veterinary Practice
200	2016	October	Gutenfelsstr. 14, 13129 Berlin	52.6005441	13.4493462	Veterinary Practice
203	2016	October	Freischuetzstr., 13129 Berlin	52.6023802	13.45136	Veterinary Practice
114	2017	April	Togostr. 45, 13351 Berlin	52.55369	13.33934	Veterinary Practice
116	2017	March	Alt-Tegel 47c, 13507 Berlin	52.58759	13.27552	Veterinary Practice
117	2017	May	Aroser Allee 111, 13407 Berlin	52.56659	13.35125	Veterinary Practice
119	2017	May	Ghanastr. 27, 13351 Berlin	52.56047	13.32984	Veterinary Practice
120	2017	July	Altglienike Feldweg	52.3975145	13.5554986	Veterinary Practice
141	2017	May	Tierpark, Berlin	52.5023038	13.5313559	IZW
142	2017	July	Volkspark Prenzlauerberg, Berlin	52.53564	13.4634372	IZW
143	2017	June	Tiergarten, Berlin	52.5144898	13.3500906	IZW
144	2017	May	Tierpark, Berlin	52.5023038	13.5313559	IZW
146	2017	May	Tierpark, Berlin	52.5023038	13.5313559	IZW
147	2017	June	Tiergarten, Berlin	52.5144898	13.3500906	IZW
149	2017	May	Tierpark, Berlin	52.5023038	13.5313559	IZW
150	2017	June	Buergerpark Pankow-Berlin	52.5694584	13.394732	IZW
152	2017	June	Tiergarten, Berlin	52.5144898	13.3500906	IZW
153	2017	June	Volkspark Prenzlauerberg, Berlin	52.53564	13.4634372	IZW

154	2017	May	Tierpark, Berlin	52.5023038	13.5313559	IZW
156	2017	June	Buergerpark Pankow-Berlin	52.5694584	13.394732	IZW
157	2017	NA	Treptower Park	52.48846	13.46974	IZW
158	2017	July	Hans-Baluschek-Park, 10829 Berlin	52.4644359	13.3570684	IZW
159	2017	July	Prenzlauerberg	52.54114	13.44009	IZW
161	2017	July	Hans-Baluschek-Park, 10829 Berlin	52.4644359	13.3570684	IZW
165	2017	May	Tierpark, Berlin	52.5023038	13.5313559	IZW
166	2017	May	Tiergarten, Berlin	52.5144898	13.3500906	IZW
167	2017	June	Tiergarten, Berlin	52.5144898	13.3500906	IZW
168	2017	July	Volkspark Prenzlauerberg, Berlin	52.53564	13.4634372	IZW
169	2017	July	Hans-Baluschek-Park, 10829 Berlin	52.4644359	13.3570684	IZW
170	2017	June	Volkspark Prenzlauerberg, Berlin	52.53564	13.4634372	IZW
172	2017	July	Volkspark Prenzlauerberg, Berlin	52.53564	13.4634372	IZW
174	2017	May	Tierpark, Berlin	52.5023038	13.5313559	IZW
175	2017	July	Volkspark Prenzlauerberg, Berlin	52.53564	13.4634372	IZW
305	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
306	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
307	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
308	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
311	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
312	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
314	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
315	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
317	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
318	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
319	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
320	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
321	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
322	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
324	2017	September	Eisenacher Str.,12629 Berlin	52.5402405	13.5902187	Pound
326	2017	October	Zum Erlenbruch, 15344 Strausberg	52.5680305	13.8773122	Pound
328	2017	September	Warnemünder Str. 18, 13059 Berlin	52.57423	13.50583	Pound
329	2017	September	Warnemünder Str. 18, 13059 Berlin	52.57423	13.50583	Pound
330	2017	September	Warnemünder Str. 18, 13059 Berlin	52.57423	13.50583	Pound
333	2017	September	12623 Berlin	52.5032943	13.6073142	Pound
334	2017	September	12623 Berlin	52.5032943	13.6073142	Pound
335	2017	September	12623 Berlin	52.5032943	13.6073142	Pound
337	2017	October	KGA Märchenland, 13089 Berlin	52.5749069	13.4650705	Pound
338	2017	November	Belziger Ring 36, 12689 Berlin	52.56367	13.57531	Pound
340	2017	October	Mahlerstraße, 13088 Berlin	52.5468372	13.4537713	Pound

341	2017	October	Kleingartenanlage 750 Jahre Berlin, 13057 Berlin	52.5739373	13.5438938	Pound
342	2017	September	Tierpark, Berlin	52.5023038	13.5313559	IZW
344	2017	September	IZW Garten, Berlin	52.50597	13.52148	IZW
345	2017	October	Treptower Park	52.48846	13.46974	IZW
346	2017	August	Treptower Park	52.48846	13.46974	IZW
348	2017	August	Treptower Park	52.48846	13.46974	IZW
349	2017	August	Treptower Park	52.48846	13.46974	IZW
350	2017	August	Treptower Park	52.48846	13.46974	IZW
113	2017	April	Choise-le-Roi-Str. 3, Berlin	52.65611	13.19784	Veterinary Practice
118	2017	April	Vielitzsee Ortsteil Strubensee, 16835	52.9397147	13.021689	Veterinary Practice
309	2017	August	Nordbahnhof	52.5318835	13.3883826	IZW
310	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
313	2017	August	Tierpark, Berlin	52.5023038	13.5313559	IZW
343	2017	September	Tierpark, Berlin	52.5023038	13.5313559	IZW
220	2017	April	Friedenstr., Berlin ?	52.5231715	13.4339728	Pound
231	2016	October	Friedenstr. 8, 16356 Ahrensfelde	52.58345	13.57986	Pound
235	2016	August	Glasberger Str. 43, 12555 Berlin	52.46971	13.57587	Pound
241	2016	September	Glambecker Ring 4, 12679 Berlin	52.55203	13.57497	Pound
243	2016	April	Zeuthen	52.3476518	13.6207615	Pound
248	2016	September	13053 Berlin	52.5559059	13.5055018	Pound
252	2016	October	Friedenstr., 16356 Ahrensfelde	52.5870868	13.5811324	Pound
257	2017	June	Dietrichstr. 5, 16356 Ahrensfelde	52.5975636	13.5557275	Pound
261	2016	September	Wolfshofstr. 25, 13591 Berlin	52.54398	13.1606	Veterinary Practice
300	2017	September	Kastanienallee 122/126, 12627 Berlin-Hellersdorf	52.543	13.60164	Pound
A1.317	2017	May	Treptower Park	52.4884599	13.4697445	IZW
A10.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A11.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A12.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A13.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A14.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A15.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A16.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A2.317	2017	June	Treptower Park	52.4884599	13.4697445	IZW
A20.038	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A21.038	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A22.038	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A23.038	2016	August	Treptower Park	52.4884599	13.4697445	IZW
A27.078	2016	June	Treptower Park	52.4884599	13.4697445	IZW
A28.078	2016	June	Treptower Park	52.4884599	13.4697445	IZW
A3.317	2017	July	Treptower Park	52.4884599	13.4697445	IZW
A30.078	2016	June	Treptower Park	52.4884599	13.4697445	IZW
A31.078	2016	June	Treptower Park	52.4884599	13.4697445	IZW
A32.078	2016	June	Treptower Park	52.4884599	13.4697445	IZW
A34.078	2016	June	Treptower Park	52.4884599	13.4697445	IZW

A35.088	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A37.088	2016	July	Treptower Park	52.4884599	13.4697445	IZW
A4.317	2017	June	Treptower Park	52.4884599	13.4697445	IZW
A43.088	2016	October	Treptower Park	52.4884599	13.4697445	IZW
A47.098	2015	August	Treptower Park	52.4884599	13.4697445	IZW
A5.317	2017	June	Treptower Park	52.4884599	13.4697445	IZW
A56.098	2015	September	Treptower Park	52.4884599	13.4697445	IZW
A59.108	2015	August	Treptower Park	52.4884599	13.4697445	IZW
A61.108	2015	August	Treptower Park	52.4884599	13.4697445	IZW
A62.108	2015	August	Treptower Park	52.4884599	13.4697445	IZW
A68.108	2015	September	Treptower Park	52.4884599	13.4697445	IZW
A9.028	2016	July	Treptower Park	52.4884599	13.4697445	IZW



Supplementary Figure 1.2: Pairwise relatedness after Queller and Goodman (QGEst) of sampled genotypes (Ind1 and Ind2) before (left) and after (right) removing related genotypes r >

Supplementary Table 1.2: Unique genotypes (N = 143) of hedgehogs in Berlin across 10 loci

ID	EEU 1	EEU 1	EEU 2	EEU 2	EEU 3	EEU 3	EEU 4	EEU 4	EEU 5	EEU 5	EEU 6	EEU 6	EEU12 H	EEU12 H	EEU36 H	EEU36 H	EEU37 H	EEU37 H	EEU43 H	EEU43 H
110	135	139	257	257	145	153	148	148	113	129	145	145	91	97	246	248	154	158	282	284
127	135	135	257	257	145	163	160	160	113	135	145	145	95	97	240	276	154	158	282	282
128	129	129	259	267	149	165	148	160	115	129	145	145	97	97	246	276	154	170	282	286
129	135	143	259	267	149	163	160	170	113	135	145	155	91	97	236	240	154	154	282	282
135	135	139	259	279	149	153	156	158	113	113	145	145	91	91	246	260	154	156	282	286
136	129	139	267	269	145	165	148	148	115	129	145	145	91	97	246	276	154	154	282	282
137	135	135	261	267	131	163	158	160	109	113	145	145	91	91	248	248	154	154	282	282
138	135	139	261	273	163	163	158	160	109	113	145	145	91	91	248	248	154	156	282	284
139	131	135	269	269	145	155	144	158	109	113	145	145	91	97	240	246	154	158	282	290
140	135	139	267	281	145	153	148	148	115	129	145	145	91	97	24	246	156	156	282	290
184	129	131	267	269	145	145	154	160	109	129	145	145	91	91	240	248	148	158	282	292
185	135	137	269	271	153	169	146	152	109	113	145	159	91	97	256	256	150	154	282	292
186	139	139	265	267	155	163	148	152	113	127	145	145	97	97	256	256	150	154	284	290
187	137	139	257	273	149	169	148	164	113	115	145	159	91	97	238	248	158	158	282	282
188	129	139	257	269	153	179	160	170	113	129	145	145	91	95	240	256	152	154	282	290
189	135	135	267	267	153	153	148	156	113	113	145	155	91	91	240	256	156	158	282	282
191	129	129	267	267	153	169	152	152	113	113	145	145	91	97	256	256	154	158	282	282
192	135	139	257	259	159	179	146	160	107	113	145	147	91	97	246	256	148	154	284	290
193	129	139	269	277	169	169	160	164	113	113	145	145	91	97	240	256	154	158	282	292
125	129	139	267	269	153	163	148	150	113	115	145	145	91	91	240	248	154	156	282	282
126	129	129	267	269	145	163	148	148	113	115	145	159	91	91	240	246	154	170	282	282
134	135	139	267	275	149	163	148	150	113	115	145	145	95	97	240	248	154	154	282	286
176	131	135	259	259	153	159	148	164	113	113	145	157	91	97	244	246	150	154	282	282
179	131	131	259	259	153	153	148	150	113	113	145	147	91	97	244	248	156	156	282	282
182	129	139	267	279	153	153	160	160	107	135	145	145	91	91	246	272	154	154	282	282
194	139	139	257	267	145	159	148	160	113	123	145	145	97	97	240	256	154	156	282	286
196	139	139	259	267	145	179	148	160	107	123	145	145	91	97	238	256	154	154	282	292
197	131	139	269	277	153	163	148	158	107	113	145	155	91	91	248	256	154	158	282	282
198	129	137	259	267	163	165	148	158	109	113	145	145	97	97	248	248	154	158	282	286
199	131	131	257	257	153	153	148	148	113	125	145	145	91	91	250	250	158	158	284	284
200	129	137	269	273	149	169	148	154	107	115	145	145	97	97	240	248	158	158	282	282
203	137	139	265	273	149	163	148	148	113	127	145	145	97	97	248	256	154	158	282	284
114	131	139	259	269	149	153	158	160	113	113	145	145	91	97	240	248	154	158	282	292
116	135	135	263	265	159	177	148	160	113	129	145	145	95	97	238	256	154	154	282	284
117	135	139	267	271	145	169	150	170	113	123	145	145	97	97	248	248	152	158	280	282
119	131	139	263	273	145	149	152	160	113	139	145	145	91	95	250	252	152	156	282	290
120	135	139	269	281	149	169	148	160	115	139	145	147	91	91	236	240	148	152	282	292
141	129	139	257	263	153	163	148	158	121	129	145	155	91	97	246	246	148	158	282	290
142	135	135	263	273	153	163	148	158	113	115	145	145	91	95	240	248	154	156	282	282
143	129	129	257	259	153	159	150	150	109	113	145	145	91	97	240	252	158	158	284	292
144	129	129	257	267	153	179	148	158	113	129	145	155	97	97	246	246	148	154	282	290
146	129	129	257	267	153	179	148	148	113	129	145	155	97	97	240	246	154	154	282	290
147	139	139	257	271	131	159	156	170	115	115	145	155	91	97	278	278	156	156	282	282
149	129	139	271	271	153	153	158	160	113	129	145	145	93	95	246	246	148	154	290	292
150	131	135	257	265	159	163	158	164	115	121	145	145	97	97	256	272	152	154	282	282

152	139	139	269	269	153	153	148	148	113	113	145	145	97	97	240	256	154	156	282	282
153	135	139	267	269	145	163	156	158	113	113	145	155	91	95	240	272	152	156	282	284
154	129	139	265	267	163	179	148	160	113	113	145	155	91	97	246	246	148	158	282	292
156	135	139	267	267	153	163	148	164	109	113	145	145	91	97	240	248	154	156	282	282
157	129	131	259	267	145	179	158	158	115	115	145	153	91	95	248	274	152	154	282	290
158	135	135	273	275	149	165	166	170	113	113	145	155	95	97	248	256	154	154	282	282
159	135	139	275	275	149	165	148	160	115	129	145	145	91	91	248	248	156	158	282	284
161	129	139	267	279	149	153	158	158	113	113	145	155	91	91	236	240	152	158	276	286
165	129	129	257	265	153	163	158	160	113	129	145	145	97	97	246	246	154	154	282	282
166	139	139	269	273	153	163	148	148	113	113	155	155	91	91	240	256	154	154	282	282
167	139	139	257	271	131	175	170	170	113	115	145	155	97	97	278	278	156	156	282	282
168	139	139	267	269	145	165	158	158	113	115	145	155	91	91	236	240	154	156	282	282
169	131	135	257	257	145	145	158	170	113	121	145	145	91	91	248	256	154	154	282	282
170	131	135	269	269	145	153	158	160	113	113	145	155	95	97	248	248	154	156	282	282
172	135	139	261	269	145	163	150	160	115	129	145	147	91	95	240	248	154	156	282	290
174	129	129	267	281	145	153	148	148	113	129	145	155	91	97	240	240	152	154	282	282
175	135	139	261	273	153	163	158	170	113	115	145	155	91	91	248	248	156	156	282	282
305	129	139	257	265	153	163	148	152	113	113	145	155	97	97	246	246	148	154	290	292
306	129	129	257	271	153	153	148	148	113	129	145	155	97	97	246	246	148	148	282	290
307	129	129	265	273	159	163	148	160	113	113	145	145	97	97	240	246	148	158	282	292
308	131	135	261	273	145	159	148	158	113	129	145	145	91	97	240	244	154	158	282	282
311	135	135	267	273	159	181	144	148	113	113	145	145	91	97	240	248	154	158	282	282
312	129	139	257	265	153	163	148	160	113	113	145	155	97	97	246	246	148	154	282	292
314	131	139	261	265	163	163	148	160	113	113	145	145	91	97	246	250	156	158	282	292
315	135	139	265	267	163	163	158	158	113	113	145	145	91	97	246	246	154	154	292	292
317	131	139	267	267	165	165	148	160	113	129	145	145	91	97	240	240	154	158	282	282
318	129	135	265	267	163	179	146	148	113	129	145	155	97	97	246	246	148	154	282	282
319	129	131	257	271	153	153	148	158	113	129	145	145	95	97	246	246	148	158	282	290
320	129	129	257	263	153	163	148	160	113	115	145	145	91	91	246	256	154	154	282	282
321	129	135	267	273	159	179	148	148	113	129	145	155	97	97	240	246	154	158	282	282
322	129	135	261	265	145	163	158	160	113	129	145	145	97	97	240	246	148	154	282	292
324	135	139	271	273	149	163	148	160	113	115	145	147	91	97	240	250	156	156	282	282
326	139	139	267	267	131	145	148	160	113	129	145	145	91	91	240	248	156	160	282	290
328	135	139	257	267	149	153	148	150	113	113	145	145	91	95	248	256	148	152	282	286
329	139	139	267	267	153	153	150	160	113	115	145	145	91	91	240	248	148	154	284	286
330	135	139	0	0	149	153	150	150	113	115	145	145	91	91	240	256	148	152	282	284
333	139	141	257	267	153	163	148	160	113	129	145	145	91	97	240	256	150	154	282	290
334	133	133	265	271	145	159	148	170	113	113	145	145	91	97	256	256	150	158	282	282
335	135	139	267	267	153	159	148	160	113	113	145	145	91	95	240	256	154	154	284	284
337	139	139	257	259	153	159	148	158	107	113	145	159	95	95	240	244	160	172	282	282
338	131	143	259	265	145	153	150	158	113	115	145	145	91	97	240	258	158	158	282	296
340	139	139	267	267	163	165	148	158	113	115	145	155	91	95	248	248	152	156	282	290
341	133	139	257	269	145	171	158	164	107	113	145	145	91	97	240	256	154	156	282	282
342	139	139	257	265	153	163	152	160	113	113	145	145	91	97	246	246	154	154	292	292
344	129	131	257	257	153	153	148	148	113	113	145	155	95	97	246	246	148	158	282	290
345	131	135	259	273	165	173	158	160	113	115	145	145	91	97	246	256	152	154	282	282
346	131	135	273	273	173	173	160	160	109	113	145	155	91	95	256	256	154	156	282	282
348	133	139	269	269	165	173	160	160	107	123	145	145	91	91	248	248	154	156	282	290
349	129	135	267	273	145	149	158	160	107	113	145	147	91	93	248	274	154	154	282	282
350	129	131	267	273	145	173	160	160	113	113	145	153	93	95	256	274	154	154	282	282

113	135	139	257	269	153	171	148	160	113	131	145	145	91	91	244	280	162	164	282	282
118	133	139	257	267	145	145	160	164	113	129	145	155	97	97	240	248	158	158	282	284
309	135	139	267	271	145	153	160	160	113	123	145	145	91	91	256	256	154	156	292	292
310	129	135	269	273	145	163	148	160	113	113	145	145	91	97	248	256	154	170	286	292
313	129	129	257	267	153	179	158	160	129	129	145	145	97	97	246	246	154	154	282	282
343	129	131	267	267	131	179	156	158	115	115	145	145	91	97	246	272	154	156	286	292
220	135	135	257	267	153	159	148	148	113	113	145	147	91	97	240	258	158	162	282	282
231	139	139	259	263	153	153	148	158	119	119	145	155	91	91	240	256	160	160	282	282
235	129	139	263	269	153	153	158	160	113	127	145	155	91	91	240	248	156	158	282	286
241	129	131	257	257	149	153	146	146	107	109	145	145	91	91	240	246	152	154	286	286
243	135	139	257	259	145	155	148	160	113	115	145	145	97	97	246	246	154	156	282	286
248	131	135	257	269	145	159	160	160	109	109	147	155	97	97	240	248	154	154	282	282
252	129	129	259	269	149	173	158	158	121	121	145	145	93	97	236	250	160	160	282	290
257	131	139	267	271	153	175	148	160	113	113	145	145	91	91	240	248	152	160	282	284
261	129	139	269	269	145	163	160	160	107	109	145	145	91	97	240	248	154	156	290	290
300	131	135	263	267	153	179	146	148	113	115	145	159	97	97	256	258	152	158	282	282
A1_317	129	135	267	273	149	149	160	160	107	113	145	147	91	93	248	274	154	156	282	282
A10_028	129	135	263	269	163	173	160	162	113	115	145	145	95	97	256	276	152	154	290	292
A11_028	131	131	259	259	153	153	156	160	113	121	145	155	91	95	240	256	154	154	282	282
A12_028	129	135	259	267	165	179	148	160	115	115	145	155	91	93	256	272	154	154	292	292
A13_028	131	135	281	281	149	153	158	160	113	121	145	145	93	95	240	240	154	154	282	282
A14_028	135	135	273	281	149	173	156	160	121	127	145	145	91	91	256	272	154	156	282	286
A15_028	129	135	269	269	173	173	160	160	115	115	145	145	97	97	246	248	154	154	282	284
A16_028	133	139	269	279	165	173	160	160	107	121	145	145	91	91	248	248	154	156	282	290
A2_317	131	135	259	267	165	165	158	160	113	115	145	145	91	97	240	276	152	154	282	282
A20_038	129	131	259	273	149	179	160	160	113	113	145	147	91	91	246	274	152	154	282	290
A21_038	129	135	259	259	149	149	160	168	107	107	145	145	97	97	250	250	154	154	284	290
A22_038	129	131	263	273	149	153	158	166	107	107	145	147	95	97	256	274	152	154	282	282
A25_078	131	135	259	269	173	179	156	160	113	115	145	145	91	97	246	246	152	154	282	290
A27_078	131	135	263	267	163	163	160	160	115	127	145	145	93	97	246	256	154	154	286	290
A28_078	131	133	263	269	153	173	148	156	107	115	145	145	93	97	246	272	154	154	282	282
A3_317	135	139	267	267	159	163	148	160	107	115	145	155	91	91	274	278	148	156	282	290
A30_078	139	139	273	273	173	173	160	160	107	113	145	145	95	97	240	256	154	156	282	282
A31_078	131	133	269	273	173	173	148	160	107	113	145	145	97	97	246	246	154	154	282	282
A32_07	131	131	267	273	173	173	156	160	113	115	145	145	91	97	246	256	152	156	282	282

8																				
A34_078	135	139	267	269	163	173	156	158	113	115	145	145	97	97	278	278	156	158	282	282
A35_088	135	139	267	267	159	163	160	160	113	113	145	155	91	91	246	272	154	156	282	282
A37_088	131	135	259	269	173	179	156	160	113	115	145	145	91	97	246	248	152	154	282	290
A4_317	137	137	0	0	149	149	148	148	123	125	145	145	91	91	246	246	146	148	282	282
A43_088	131	131	271	273	0	0	156	160	113	113	145	145	97	97	274	276	154	154	282	282
A47_098	131	139	269	273	173	173	160	160	107	127	145	145	97	97	240	240	154	154	282	282
A5_317	129	135	259	269	153	173	148	160	113	115	145	145	93	97	240	248	154	154	282	282
A56_098	131	135	271	273	173	179	160	168	109	113	155	155	91	91	256	256	154	156	282	282
A59_108	135	135	269	269	173	173	158	160	115	115	145	145	97	97	0	0	154	154	282	282
A61_108	131	139	267	267	159	163	152	156	107	113	145	145	91	91	256	256	154	156	290	290
A62_108	129	135	259	281	149	165	148	162	113	115	145	145	91	91	248	256	154	160	290	292
A68_108	139	139	257	267	163	163	160	160	113	113	145	145	91	91	246	272	156	156	0	0
A9_028	131	131	267	267	165	165	160	160	127	127	145	145	93	93	256	256	154	154	286	290

Missing values are indicated by '0'.

Supplementary Table 1.3: Unique unrelated genotypes (N = 65, r < 0.5) of hedgehogs in Berlin across 10 loci

ID	EEU 1	EEU1 .1	EEU 2	EEU2 .1	EEU 3	EEU3 .1	EEU 4	EEU4 .1	EEU 5	EEU5 .1	EEU 6	EEU6 .1	EEU12 H	EEU12 H.1	EEU36 H	EEU36 H.1	EEU37 H	EEU37 H.1	EEU43 H	EEU43 H.1
110	135	139	257	257	145	153	148	148	113	129	145	145	91	97	246	248	154	158	282	284
127	135	135	257	257	145	163	160	160	113	135	145	145	95	97	240	276	154	158	282	282
129	135	143	259	267	149	163	160	170	113	135	145	155	91	97	236	240	154	154	282	282
135	135	139	259	279	149	153	156	158	113	113	145	145	91	91	246	260	154	156	282	286
139	131	135	269	269	145	155	144	158	109	113	145	145	91	97	240	246	154	158	282	290
140	135	139	267	281	145	153	148	148	115	129	145	145	91	97	240	246	156	156	282	290
184	129	131	267	269	145	145	154	160	109	129	145	145	91	91	240	248	148	158	282	292
185	135	137	269	271	153	169	146	152	109	113	145	159	91	97	256	256	150	154	282	292
188	129	139	257	269	153	179	160	170	113	129	145	145	91	95	240	256	152	154	282	290
191	129	129	267	267	153	169	152	152	113	113	145	145	91	97	256	256	154	158	282	282
192	135	139	257	259	159	179	146	160	107	113	145	147	91	97	246	256	148	154	284	290
193	129	139	269	277	169	169	160	164	113	113	145	145	91	97	240	256	154	158	282	292
134	135	139	267	275	149	163	148	150	113	115	145	145	95	97	240	248	154	154	282	286
194	139	139	257	267	145	159	148	160	113	123	145	145	97	97	240	256	154	156	282	286
196	139	139	259	267	145	179	148	160	107	123	145	145	91	97	238	256	154	154	282	292
198	129	137	259	267	163	165	148	158	109	113	145	145	97	97	248	248	154	158	282	286
199	131	131	257	257	153	153	148	148	113	125	145	145	91	91	250	250	158	158	284	284
114	131	139	259	269	149	153	158	160	113	113	145	145	91	97	240	248	154	158	282	292
116	135	135	263	265	159	177	148	160	113	129	145	145	95	97	238	256	154	154	282	284
117	135	139	267	271	145	169	150	170	113	123	145	145	97	97	248	248	152	158	280	282
119	131	139	263	273	145	149	152	160	113	139	145	145	91	95	250	252	152	156	282	290
120	135	139	269	281	149	169	148	160	115	139	145	147	91	91	236	240	148	152	282	292
143	129	129	257	259	153	159	150	150	109	113	145	145	91	97	240	252	158	158	284	292
150	131	135	257	265	159	163	158	164	115	121	145	145	97	97	256	272	152	154	282	282
152	139	139	269	269	153	153	148	148	113	113	145	145	97	97	240	256	154	156	282	282
153	135	139	267	269	145	163	156	158	113	113	145	155	91	95	240	272	152	156	282	284
157	129	131	259	267	145	179	158	158	115	115	145	153	91	95	248	274	152	154	282	290
158	135	135	273	275	149	165	166	170	113	113	145	155	95	97	248	256	154	154	282	282
159	135	139	275	275	149	165	148	160	115	129	145	145	91	91	248	248	156	158	282	284
161	129	139	267	279	149	153	158	158	113	113	145	155	91	91	236	240	152	158	276	286
168	139	139	267	269	145	165	158	158	113	115	145	155	91	91	236	240	154	156	282	282
169	131	135	257	257	145	145	158	170	113	121	145	145	91	91	248	256	154	154	282	282
170	131	135	269	269	145	153	158	160	113	113	145	155	95	97	248	248	154	156	282	282
172	135	139	261	269	145	163	150	160	115	129	145	147	91	95	240	248	154	156	282	290
324	135	139	271	273	149	163	148	160	113	115	145	147	91	97	240	250	156	156	282	282
333	139	141	257	267	153	163	148	160	113	129	145	145	91	97	240	256	150	154	282	290
334	133	133	265	271	145	159	148	170	113	113	145	145	91	97	256	256	150	158	282	282
337	139	139	257	259	153	159	148	158	107	113	145	159	95	95	240	244	160	172	282	282
338	131	143	259	265	145	153	150	158	113	115	145	145	91	97	240	258	158	158	282	296
340	139	139	267	267	163	165	148	158	113	115	145	155	91	95	248	248	152	156	282	290
341	133	139	257	269	145	171	158	164	107	113	145	145	91	97	240	256	154	156	282	282
113	135	139	257	269	153	171	148	160	113	131	145	145	91	91	244	280	162	164	282	282
118	133	139	257	267	145	145	160	164	113	129	145	155	97	97	240	248	158	158	282	284
309	135	139	267	271	145	153	160	160	113	123	145	145	91	91	256	256	154	156	292	292
310	129	135	269	273	145	163	148	160	113	113	145	145	91	97	248	256	154	170	286	292

343	129	131	267	267	131	179	156	158	115	115	145	145	91	97	246	272	154	156	286	292
231	139	139	259	263	153	153	148	158	119	119	145	155	91	91	240	256	160	160	282	282
235	129	139	263	269	153	153	158	160	113	127	145	155	91	91	240	248	156	158	282	286
241	129	131	257	257	149	153	146	146	107	109	145	145	91	91	240	246	152	154	286	286
243	135	139	257	259	145	155	148	160	113	115	145	145	97	97	246	246	154	156	282	286
248	131	135	257	269	145	159	160	160	109	109	147	155	97	97	240	248	154	154	282	282
252	129	129	259	269	149	173	158	158	121	121	145	145	93	97	236	250	160	160	282	290
300	131	135	263	267	153	179	146	148	113	115	145	159	97	97	256	258	152	158	282	282
A10_028	129	135	263	269	163	173	160	162	113	115	145	145	95	97	256	276	152	154	290	292
A11_028	131	131	259	259	153	153	156	160	113	121	145	155	91	95	240	256	154	154	282	282
A12_028	129	135	259	267	165	179	148	160	115	115	145	155	91	93	256	272	154	154	292	292
A13_028	131	135	281	281	149	153	158	160	113	121	145	145	93	95	240	240	154	154	282	282
A14_028	135	135	273	281	149	173	156	160	121	127	145	145	91	91	256	272	154	156	282	286
A20_038	129	131	259	273	149	179	160	160	113	113	145	147	91	91	246	274	152	154	282	290
A21_038	129	135	259	259	149	149	160	168	107	107	145	145	97	97	250	250	154	154	284	290
A22_038	129	131	263	273	149	153	158	166	107	107	145	147	95	97	256	274	152	154	282	282
A3_317	135	139	267	267	159	163	148	160	107	115	145	155	91	91	274	278	148	156	282	290
A4_317	137	137	0	0	149	149	148	148	123	125	145	145	91	91	246	246	146	148	282	282
A61_108	131	139	267	267	159	163	152	156	107	113	145	145	91	91	256	256	154	156	290	290
A62_108	129	135	259	281	149	165	148	162	113	115	145	145	91	91	248	256	154	160	290	292

2 Supplementary information Chapter 2: Application of an SSR-GBS marker system on an investigation of European Hedgehog species and their hybrid zone dynamics

Data Accessibility: Raw reads from the low-coverage whole-genome sequencing libraries used for marker development can be found in the Sequence Read Archive (SRA) under the reference PRJNA495814. The SSR allele sequences were submitted to GenBank and can be found with the reference numbers MH683170-MH683548.

Supplementary Table 2.1: Samples used with information of location of origin, species identification, starting material for DNA isolation, institution providing the sample, and coordinates. For some samples coordinates were not available (NA).

Sample Name	Region	Species	Material	Material origin	Coordinate
36516	Berlin	*E. europaeus*	tissue	Leibniz Institute for Zoo and Wildlife Research	NA
2014429	Bavaria	*E. europaeus*	tissue	Biologiezentrum Linz	48.268194, 13.034333
2014430	Bavaria	*E. europaeus*	tissue	Biologiezentrum Linz	48.268194, 13.034333
200689	Czech Republic	*E. europaeus*	tissue	Biologiezentrum Linz	48.972278, 14.473722
2006603	Czech Republic	*E. europaeus*	tissue	Biologiezentrum Linz	48.735556, 14.491667
2008188	Czech Republic	*E. europaeus*	tissue	Biologiezentrum Linz	48.814528, 14.284139
20111185	Czech Republic	*E. europaeus*	tissue	Biologiezentrum Linz	48.846472, 14.455722
20111186	Czech Republic	*E. europaeus*	tissue	Biologiezentrum Linz	48.786028, 14.456222
2004247	West Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.343861, 14.720444
2005615	West Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.243000, 14.849889
2006606	West Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.358806, 14.511944
2007102	West Linz	*E. europaeus*	tissue	Biologiezentrum Linz	NA
2012159	West Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.141556, 13.735889
2016172	West Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.336861, 14.435667
2002243	Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.337944, 14.311972
2014439	Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.336806, 14.313083
2014456	Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.331750, 14.312806
2014839	Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.334111, 14.326778
2015786	Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.334111, 14.326778
200695	Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.334111, 14.326778
200792	Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.332389, 14.312806
2008219	East Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.345444, 14.511917
2014438	East Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.254750, 14.424056
2014581	East Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.351444, 14.092444
2014683	East Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.322167, 14.194278
2016171	East Linz	*E. europaeus*	tissue	Biologiezentrum Linz	48.361806, 14.490889
2014445	West Vienna	*E. europaeus*	tissue	Biologiezentrum Linz	48.126806, 14.595306
IBK1	Innsbruck	*E. europaeus*	saliva	shelter Innsbruck	NA
IBK2	Innsbruck	*E. europaeus*	saliva	shelter Innsbruck	NA
IBK3	Innsbruck	*E. europaeus*	saliva	shelter Innsbruck	NA
IBK4	Innsbruck	*E. europaeus*	saliva	shelter Innsbruck	NA

IBK5	Innsbruck	*E. europaeus*	saliva	shelter Innsbruck	NA
IBK6	Innsbruck	*E. europaeus*	saliva	shelter Innsbruck	NA
IBK7	Innsbruck	*E. europaeus*	saliva	shelter Innsbruck	NA
VA25	Voralberg	*E. europaeus*	saliva	Sheter Bludenz	NA
VA26	Voralberg	*E. europaeus*	saliva	Sheter Bludenz	NA
VA27	Voralberg	*E. europaeus*	saliva	Sheter Bludenz	NA
VA28	Voralberg	*E. europaeus*	saliva	Sheter Bludenz	NA
VA29	Voralberg	*E. europaeus*	saliva	Sheter Bludenz	NA
VA30	Voralberg	*E. europaeus*	saliva	Sheter Bludenz	NA
VA31	Voralberg	*E. europaeus*	saliva	Sheter Bludenz	NA
2008174	Slovakia	*E. roumanicus*	tissue	Biologiezentrum Linz	48.669667, 17.782222
2008176	Slovakia	*E. roumanicus*	tissue	Biologiezentrum Linz	48.619194, 20.634222
2014420	Croacia	*E. roumanicus*	tissue	Biologiezentrum Linz	47.770000, 16.801306
2014417	Hungary	*E. roumanicus*	tissue	Biologiezentrum Linz	47.629778, 16.636306
201463	Macedonia	*E. roumanicus*	tissue	Biologiezentrum Linz	44.061111, 18.589667
200674	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.227417, 14.600472
2006187	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.203833, 14.731222
2008185	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.338028, 14.298750
2009167	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.343083, 14.702861
2014565	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.250528, 14.580056
2014582	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.215444, 14.449528
2014837	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.228361, 14.528750
2016168	West Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.189111, 14.696389
200669	Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.298472, 14.303611
200675	Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.279750, 14.389139
2006613	Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.282972, 14.287611
2008184	Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	47.874333, 16.945667
2014425	Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.327444, 14.327389
2016169	Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.313000, 14.276139
2012154	East Vienna	*E. roumanicus*	tissue	Biologiezentrum Linz	48.279056, 16.635917
2012155	East Vienna	*E. roumanicus*	tissue	Biologiezentrum Linz	48.279056, 16.635917
04NHMro	East Vienna	*E. roumanicus*	tissue	Natural History Museum	NA
2008186	Southeast Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.029833, 14.189528
2014427	Southeast Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.042667, 13.989028
2014838	Southeast Linz	*E. roumanicus*	tissue	Biologiezentrum Linz	48.202389, 14.119889
2008182	Neusidlesee	*E. roumanicus*	tissue	Biologiezentrum Linz	46.435667, 15.904111
2013168	Neusidlesee	*E. roumanicus*	tissue	Biologiezentrum Linz	47.723167, 16.867111
2014421	Neusidlesee	*E. roumanicus*	tissue	Biologiezentrum Linz	47.770000, 16.801306
2014422	Neusidlesee	*E. roumanicus*	tissue	Biologiezentrum Linz	47.742889, 16.832861
2014423	Neusidlesee	*E. roumanicus*	tissue	Biologiezentrum Linz	48.206361, 14.380111
2015109	Burgenland	*E. roumancius*	tissue	Biologiezentrum Linz	47.060361, 16.315056
KLF66	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF67	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF70	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF72	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF73	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF76	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA

KLF80	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF82	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF83	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA
KLF84	Klagenfurt	*E. roumanicus*	saliva	Shelter Klagenfurt	NA

Supplementary Table 2.2: Complete list of all primers designed with the following information: from which species they were designed (Species), in which primer mix they were included in the multiplex PCR (Mix), repetition motif (Motif), number of times it was repeated in the original sequence (Nr. Repeats), primer sequence (Forward and Reverse) and allele length variation (Amplicon length variation).

Primer Name	Species	Mix	Motif	Nr. Repeats	Forward	Reverse	Amplicon length variation
E1	*E. roumanicus*	R1	AC	16	TCATGCTAGGCACTGCTATT	AAGTGCAATCAGACCAGTGA	454 - 486
E11	*E. roumanicus*	R1	AAAG	7	ACGTTCCTCTCTGGGGAATA	TTCAAGACCCTGTTCTCCAC	428 - 460
E18	*E. roumanicus*	R1	ATTTT	9	TAGCCTGGGGGAAAATCAAG	GCAATTTCCAGTAGAGGGGA	438 - 475
E26	*E. roumanicus*	R1	CAA	10	TTAAGGAACTCAGGGTTGGG	GTGTCAATGGAAGCAAAGCT	487 - 502
E29	*E. roumanicus*	R1	TCAA	7	CTTGTGCACTGTGATGTGAG	ACGAAGTTTCCAGGAAGCTC	486 - 494
E31	*E. roumanicus*	R1	AACA	7	GGAAGCGCCTTCATTATAGC	CTCCTGTCACTAGCCAGAAG	476 - 484
E36	*E. roumanicus*	R1	GAAAG	9	ACAGTGAAGACAGGGAAGC	CTTAAAATGGCTAAGGTGGT	452 - 517
E4	*E. roumanicus*	R1	CT	13	TCAAGGAGTGTGTTGACCAG	ATCCCTTTGCTCAGCCAAT	452 - 462
E9	*E. roumanicus*	R1	ATT	13	GTTGACACTCTTTGCTGCTT	CAAGTCCTCACTAAGCCTGT	425 - 444
E10	*E. roumanicus*	R2	AAAG	11	AAGCACAACAACAATGGCAA	ACGTACTGAGCCTTTCAAGA	437 - 545
E16	*E. roumanicus*	R2	AAGAA	12	CACTAGGCAGAAAACACACG	ACCACAGATGCTGTAGACAG	425 - 480
E2	*E. roumanicus*	R2	AC	16	TGGGTAGCAGCTAAAGGAAG	GACAAAATCCTCCCTGGCTA	448 - 482
E27	*E. roumanicus*	R2	TTG	9	AGATGCTCAAGGGAAACTGA	TCACAGCATACTTAGGAGCC	452 - 477
E30	*E. roumanicus*	R2	TCAC	8	AGCGTTAAATACATCCGGCT	AACCCATTGACTCTCTGACA	430 - 458
E34	*E. roumanicus*	R2	TCTA	10	AGACCACAGTGTCCAAGTTT	GATTTCCCCTGTGTAGGTGA	428 - 466
E35	*E. roumanicus*	R2	AAAAT	6	TGGGTTGTAGATAACACTCA	ACTGCAGGTGGAGATATGTG	464 - 482
E37	*E. roumanicus*	R2	CTTTT	7	CTGCAGTTTGCTCTGGATTC	AAGAAAGAAGCCCTTGTCCA	426 - 456
E5	*E. roumanicus*	R2	GA	17	TTTCTTGCTCAGAACCCTGA	CAGGGGGAATGCTTTTCAAG	446 - 480
E7	*E. roumanicus*	R2	AAT	10	ACCATAGCTTTGTAATCTCCT	AGGATGATGGCCCTTTGAAA	445 - 463
E13	*E. roumanicus*	R3	AAGG	11	AGGTAGAAGGCAGACAATGG	TTGAAACACTGACGTAGGCT	428 - 476
E19	*E. roumanicus*	R3	GAAAT	12	CCTTGCTTGTTTCCTAAGCC	ATCACTGGGACTCCCTCAAT	472 - 527
E20	*E. roumanicus*	R3	TATTC	11	TGGATGGATGGAAAACTGAGA	GGGTGCTGATTCATCTACCT	477 - 522
E22	*E. roumanicus*	R3	AG	15	ACGGAAGGAAATACTGCCAA	CCTTCCCCTTTGTGAGAACT	470 - 502
E23	*E. roumanicus*	R3	CCT	8	GGCTATAGGCAGATTGGTGT	GAAGGTCCCAGGAACCATAG	441 - 504
E24	*E. roumanicus*	R3	GCA	7	TTCTGAGGTCTCATCTGTGC	CTGTCTGTGGTCCAGGAAAG	452 - 452
E28	*E. roumanicus*	R3	TTG	8	CCTAGTGGTAGCTTCTCACA	TTGGCTCCAGTCAAGTTCTC	440 - 452
E3	*E. roumanicus*	R3	CA	17	GGCAGACTGTCTAGTTCACA	GGTCTAGGACTGCACCATTT	476 - 512
E8	*E. roumanicus*	R3	ATT	8	CCTCCAGGAGAGATTTTGCT	CAAATGAGTGGAAGCCATGC	489 - 498
W1	*E. europaeus*	E1	AAAAT	7	GGGTAAAACAGGTCTGATGT	AAACTTGTCAGGAAGCAGTT	382 - 407
W10	*E. europaeus*	E1	AAAAC	7	ATAGCTGGATAGTGGTCTGG	ACATCTTTTCTTCCTCACAGT	398 - 433
W11	*E. europaeus*	E1	CTTC	10	AGTCACCATTCTCCACTTTC	ACCCTGAGTGAAGAAGGATA	413 - 435

W12	*E. europaeus*	E1	GAAA	8	AACTCAAATTACAAGGGGCC	TCCAATAACTAGGGGTTTAAGT	386 - 474
W13	*E. europaeus*	E1	TTTA	7	TTTCACTCTGGGTTACTGTG	AAGTGGTGCAACTCTAAGAC	386 - 395
W14	*E. europaeus*	E1	ATAG	10	AAAAGGACCTAAATGGGAGG	ACAGGGAACAAAGATGCTTA	376 - 408
W15	*E. europaeus*	E1	ATAA	8	ATACTCCCAGCCTGTTTCTA	ACCTCCCAAGAACTCTATCA	367 - 390
W16	*E. europaeus*	E1	TTAA	7	GTGTAAAGCAGTATGTTGCC	AATACAGTGTACAAGGACGC	407 - 419
W18	*E. europaeus*	E1	AATA	8	ACTCAAAAGTTTTCCACCCT	TTTTAGGCTCTGCTCTTCTG	403 - 411
W19	*E. europaeus*	E1	TTCT	13	AGAGATCAGACTAACGTTTTT	GGGGAGAATTTGGTACTGTA	402 - 443
W21	*E. europaeus*	E1	TTTA	7	ACTTCACTATCACCCTTCAA	ACTTGATTTGTTTATGGGGTG	395 - 403
W23	*E. europaeus*	E1	TGGA	13	TCTTCCCTTAAGCTACTGGA	TCTCAATTGTTTAGACATTGAGT	386 - 414
W29	*E. europaeus*	E1	CT	15	CATTACCGTGCACACAGA	GTTTGATCCCCACCACTTAA	406 - 422
W30	*E. europaeus*	E1	CT	17	TCTCATTGGATAGTGCACTG	TGCCTAATAGCAAATACACA	405 - 441
W31	*E. europaeus*	E1	GA	20	CACTTTCAATGCAGAACGTG	CAAACTGGACTAGGACAGAG	397 - 423
W32	*E. europaeus*	E1	GT	13	CAGTCAATGCATTCCCAATC	TGTGTGGTACAGGGAATAGA	415 - 451
W33	*E. europaeus*	E1	CA	11	AGAAAAGACCTCAGGAGACT	CCTGGAGAGTGGAAAAGTTA	424 - 456
W6	*E. europaeus*	E1	TTATT	7	AGGAGTTCTCAGTGATGAGA	AATACAGGCTCTGGGATAGT	378 - 404
W7	*E. europaeus*	E1	TCTTT	9	TTAGCTTGGTTTTCACAGGT	GAGTGGCAGTCTTCAAGTAG	384 - 419
W8	*E. europaeus*	E1	TTCCT	10	ATAGGAGGACTGGCGATC	AATGGAGGGAGTAGATGGG	364 - 424
W9	*E. europaeus*	E1	TTTCT	10	TTCAATCTCAAGTACCACATT	GATGCACCTGGTTGAGAG	384 - 414
E32	*E. roumanicus*	R3	ATCT	7	TGACAGTGTGTGGTTGACTT	TTCACCATCGCAGAGAACAT	Failed in Multiplex
E25	*E. roumanicus*	R1	TAC	9	TGTTATCATGCCTGAGGACC	CTGGTTGGGAAGAGAAACCT	Failed in Multiplex
E6	*E. roumanicus*	R1	AAT	16	CTCTTGGTGTGCATGACAAG	CTGTGACCCGTGTAGTTGG	Failed in Multiplex
W20	*E. europaeus*	E1	TAGA	8	TGCACATTACAATGTTCAAGG	TACATCAGGGAGAGTACAGG	Failed in Multiplex
W24	*E. europaeus*	E1	ATA	13	GCAATAATAACAAGAAGGGCA	AAGAAGTGACTGGTTTGGAG	Failed in Multiplex
W25	*E. europaeus*	E1	TTA	14	CTTTATGGGGTGCAGAAGAT	CACGATGAGCAAAGCTATTC	Failed in Multiplex
W26	*E. europaeus*	E1	TAT	15	TTTCCAGAAGATGTGGTCAG	TACAAATCTCAGCACCACTC	Failed in Multiplex
W27	*E. europaeus*	E1	ATA	9	AGCCAAAGAATAGAAGCAAGA	GCATTCTGTGGTCATGAGTA	Failed in Multiplex
W3	*E. europaeus*	E1	AAAGA	6	GAAGAAGTTTCCTCCTCTGG	GGTGGACTGAACCATTTCTT	Failed in Multiplex
W5	*E. europaeus*	E1	AAAAT	8	CACCAGGTTAAGCGTACATA	AAAAGTGCTACTAGGGAAGC	Failed in Multiplex
E12	*E. roumanicus*	Failed in single PCR	AAAG	7	AACAGAACAGCCCTGATGTT	TTGTCTTGCTTCTGGTGAGT	Failed in single PCR
E14	*E. roumanicus*	Failed in single PCR	AAGG	13	ACATGACTGTGGGTTGAGTG	CTCCAGCTCCACTGCTTTAG	Failed in single PCR
E15	*E. roumanicus*	Failed in single PCR	AAATA	7	CTGGATCAGTGAAGCTTCCA	CAAACTGGGTTAAGTGCACA	Failed in single PCR
E17	*E. roumanicus*	Failed in single PCR	ATTCC	18	ACAACCCTTCAGCTTCATCA	TAGTAGGGTGAGTCTCTGGG	Failed in single PCR
E21	*E. roumanicus*	Failed in single PCR	GA	14	TACCCATTATGCTACCCACC	TTCTGGTACATGTGCTACCG	Failed in single PCR
E33	*E. roumanicus*	Failed in single PCR	ATCT	13	CTCCATCACATGTGCCAAAG	CCACTGGCATACTACTGTGT	Failed in single PCR
W17	*E. europaeus*	Failed in single PCR	GAAA	9	TGTGATGAGGTGTTTGTTCT	AGATTTGTTGCAGGTGTCTC	Failed in single PCR
W2	*E. europaeus*	Failed in single PCR	AAATA	8	CATGAATCCACTGCTCCTAG	CTGTAGAGGTGTTGTTTTGC	Failed in single PCR
W22	*E. europaeus*	Failed in single PCR	AGGA	14	CTCATTGCAGGAAACTTCAC	GTTGTATTGTCTTATTTGGAGGT	Failed in single PCR

W28	*E. europaeus*	Failed in single PCR	AC	16	TTCTTGTTAGACCCTGAAGC	GTGACACTGGGACTCAAAC	Failed in single PCR
W34	*E. europaeus*	Failed in single PCR	AG	15	AGGGAACTGCACTATGTCTA	GCACACCTGGTTAAACACAT	Failed in single PCR
W4	*E. europaeus*	Failed in single PCR	TTTTG	7	ACTGAAGGAAGCTTCTGTG	GTAGTCTTTGAGCTTTGTGC	Failed in single PCR

Supplementary Table 2.3: Descriptions and procedures used in the in-house-scripts. We used the scripts one after the other and information concerning how this was done can be found in the material and methods. The scripts 1 to 2 can be run sequentially using the wrapper *microsatPip*.

microsatPip

File name: microsatPip.sh

Requirements: Unix, scripts 1 and 3 in the same directory of microsatPip.sh

Description: This is wrapper script that runs the programs FASTQC, PEAR, plus the scripts 1 to 3 one after the other. The results from each step are saved in a directory containing the seven folder. Initially the it runs the program FASTQC where the output is saved in the folder 'FastqcOut'. The it sorts the read according to name saving them in the directory 'Sorted'. Sorted reads are then merged with PEAR and saved in the folder 'MergedOut'. Files are renamed based on a user inputted sample sheet. Samples names should not contain any spaces or underscores. The renamed files are saved in the folder 'SeparatIn' and used for demultiplexing. Demultiplexed fastq files are saved in the folder 'SeparatOut'. Sequence lengths counts are determined and saved in one text file per sample per markers in the directory 'MarkerStatistics'. The csv codominant matrix and sequence length plots produced by script 3 are saved on the 'Markerplots' directory.

How to run: ./microsatPip.sh [1] [2] [3] [4] [5]

extract_reads_correct_primer_merged.py [1] [2] [3] [4] [5]

[1] Directory containing input fastq files.

[2] Quality threshold from which sequences should be filtered in quality control step.

[3] File containing primer information: this should be a tab separated text file containing one locus per line with the following information:

maker name[TAB]sequence primer forward[TAB] sequence reverse primer

Markers should be named in the following way: MarkerName_RepetitionMotif (ex: HH1_AT)

[4] File containing sample names information: this should be a coma separated file containing one sample per line with the following format: Name in input fastq before the first underscore , new name.

For example, for the paired reads files: P5-1-P7-1_L001_R1_001.fastq, P5-1-P7-1_L001_R2_001.fastq to be replaced with the name Sample1, this should have the following format:

P5-1-P7-1,Sample1

Script number 1

File name: primer_demultiplex.py

Requirements: python 2 or 3; Biopython

Description: This script demultiplexes merged fastq files according to primer content. The outputs are one fastq file per sample and per locus. This script allows for a user specified maximum number of mismatches between the primer and the reads. Only reads with a mismatch to both primers below to the defined are kept. In this case they are saved in a separate file. Moreover, sequences below a certain length can be excluded.

How to run: python extract_reads_correct_primer_merged.py [1] [2] [3] [4] [5]

[1] Directory containing input fastq files. Files should be named without the underscored character (ex: Sample1.fastq)

[2] File containing primer information: this should be a tab separated text file containing one locus per line with the following information:

maker name[TAB]sequence primer forward[TAB] sequence reverse primer

Markers should be named in the following way: MarkerName_RepetitionMotif (ex: HH1_AT)

[3] Maximum number of mismatches

[4] Minimum sequence length

[5] Directory to save output files. Files names are save in the following format: RepetitionMotif_SampleName_MarkerName.fasq (ex: Sample1_HH1_AT.fastq)

Script number 2

File name: CountLengths.sh

Requirements: Unix system

Description: Per fastq file it counts the number of occurrences of each sequence length present. It outputs this information in a space separated text file being the first column the length and the second the number of occurrences. Example:

417 14

422 18

418 276

423 282

How to run: sh CountLengths.sh [1] [2]

[1] Directory containing input demultiplexed fastq files. Files should be as described in the output from script 1: RepetitionMotif_SampleName_MarkerName.fasq (ex: Sample1_HH1_AT.fastq)

[2] Directory to save output files. Output file is saved in the following format: MarkerName_RepetitionMotif_SampleName_.statistics (ex: HH1_AT_Sample1_.statistics)

Script number 4

File name: extract_alleles.py

Requirements: python 2 or 3; Biopython.

Description: It extracts all sequences with the same length of the alleles saved in the csv file produced by script number 2 and saves them in a fasta file per sample and allele.

How to run: python extract_alleles_of_a_certain_length_v2.py [1] [2] [3]

[1] Coma separated text file containing genotypes. This file should contain a header containing the

marker information. The next lines it contains the genotype information. Th first column should contain the sample names while the remaining the genotype information. Two columns per markers should be added allowing for heterozygote genotypes. Missing data should be coded as 0. Example:

samplename	HH1_TA	HH1_TA	HH2_TGT	HH2_TGT	HH3_AAAC	HH4_AAAC
Sample1	425	425	429	426	418	418
Sample2	423	425	429	429	0	0
Sample3	0	0	429	429	414	414
Sample4	427	427	426	426	418	418

[2] Directory containing input fastq files. These are the output from script 1.

[3] Directory to save output fasta files. These files are named in the following format: MarkerName_SampleName_Al_SequenceLength.fasta (ex: HH1_AT_Sample1_Al_425.fasta)

Script number 5

File name: get_consensus_and_freq.py

Requirements: python 2 or 3.

Description: It produces a consensus sequence per file keeping bases above a certain similarity threshold. For positions where this is not meet the script outputs a 'N'.

How to run: python get_consensus_and_freq.py [1] [2] [3]

[1] Directory containing input fasta extracted based on length genotype information (output from script 4)

[2] Directory to save the consensus files. Files will be named in the following format: Marker Name_Sample Name_Al_Sequence Length_C Numer Of Sequences Used_Consensus Threashold.fasta (ex: HH1_AT_Sample1_Al_425_C881_70.fasta)

[3] Similarity of frequency threshold in an integer form. The value of 0.7 will do a 70% consensus.

Script 6

File name: correct_allele_sequence.py

Requirements: python 2 or 3

Description: In case a sequence has an ambiguous base ('N') after the consensus and it is homozygote based on sequence length (SL), it divides the sequence into two new ones. The Ns are corrected based on the frequency that the N position shows up in the reads extracted from script 4 taking the two most frequent nucleotide combinations. In case of heterozygote genotype based on SL the sequence is not divided but only corrected with the most frequent nucleotide information.

How to run: python correct_allele_sequence.py [1] [2] [3] [4]

[1] fasta file containing all consensus sequences from one marker. File name should be MarkerName.fasta (ex: HH1_AT.fasta). Sequences should be named in the following format: MarkerName_SampleName_Al_SequenceLength_CNumerOfSequencesUsed_ConsensusThreashold (ex: >HH1_AT_Sample1_Al_425_C881_70)

[2] Directory containing sequences extracted based on length genotypes (output from script 4)

[3] Minimum number of sequence counts required for an allele to be condidered

[4] Name of the output fasta file

Script number 7

File name: call_alleles_from_fasta.py

Requirements: python 2 or 3.

Description: Uses the haplotypes obtained from the SNP correction process and converts them into allele's numbers. If a haplotype can be assigned to more than one allele it is saved as missing data. The results are saved in tab separated text file in the format of a codominant matrix (*matrix.txt). Allele's numbers and which haplotypes they correspond to are saved in file ending with *allelle_list.txt with the following format:

Marker 1

Allele 1: Haplotype

Allele 2: Haplotype

…

Marker 2

…

How to run: python call_alleles_from_fasta.py [1] [2] [3] [4] [5]

[1] Directory containing haplotypes per locus in fasta format (output from script 7)

[2] Prefix common to all input files (ex: HH in marker HH1)

[3] List of samples names to be considered. This should be a text file with one sample name per line)

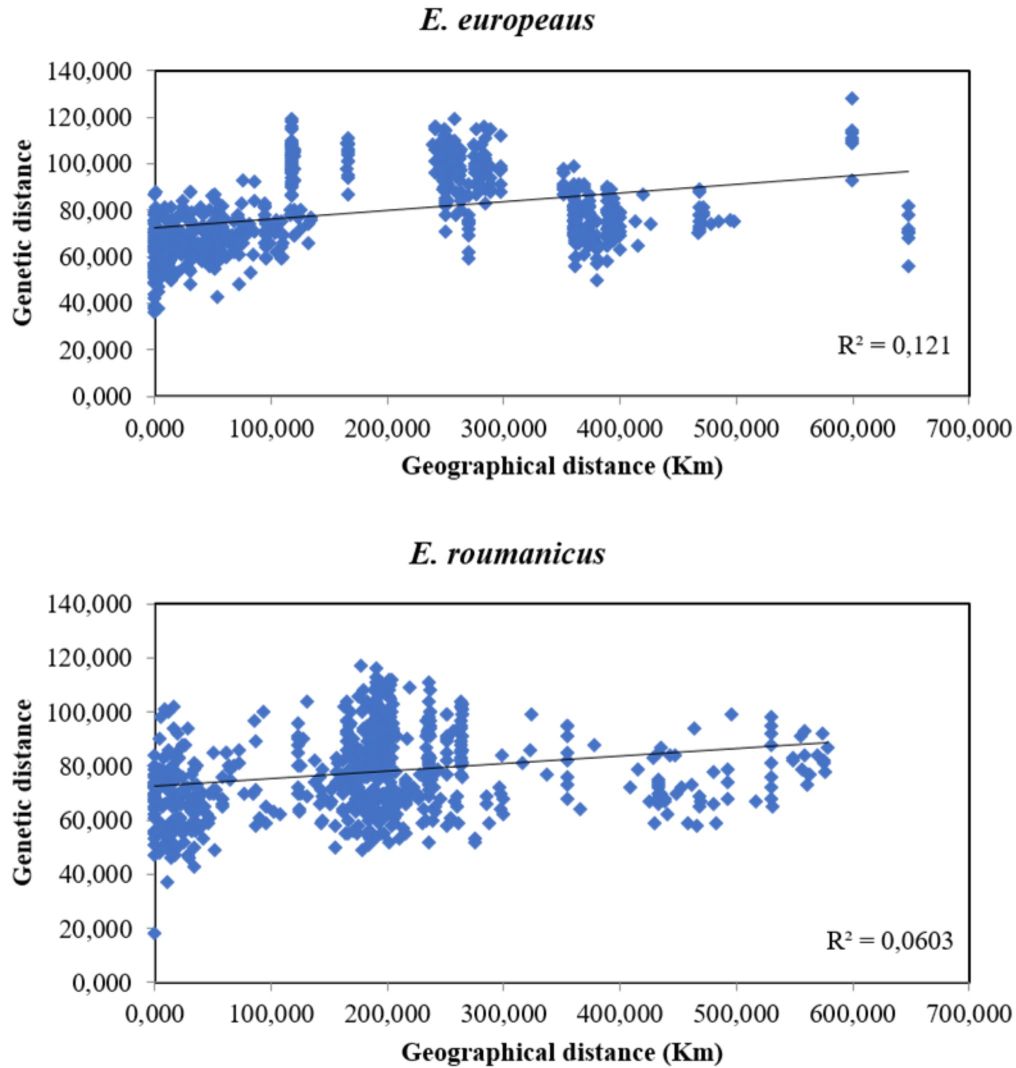
[4] Prefix that should be used to save output files

[5] Minimum number of sequences required to for a haplotype to be consider

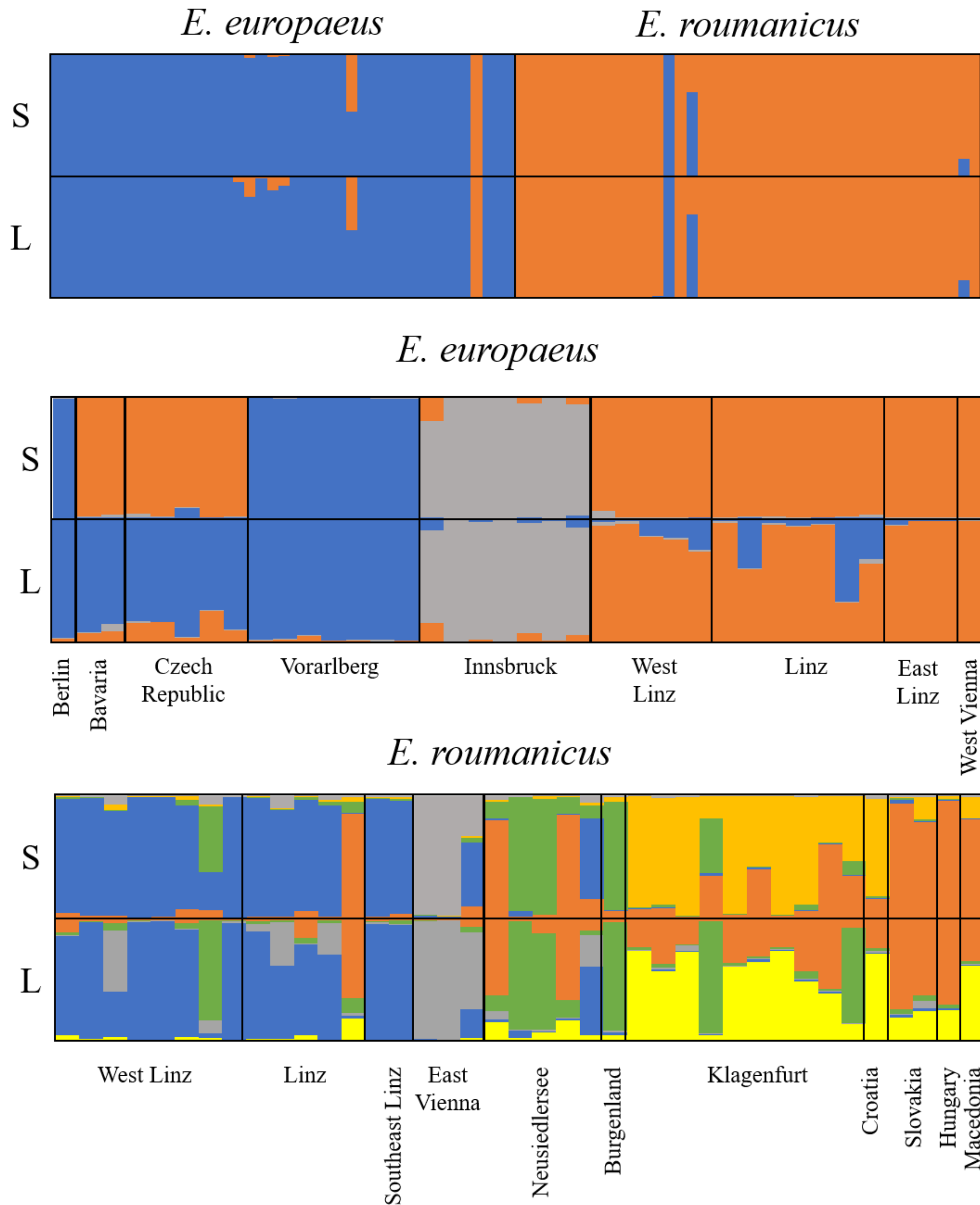
Supplementary Table 2.4: Amplification success shown as percentage of missing data and variability measures: Na – number of alleles, HO – observed heterozygosity, HE – expected heterozygosity and PIC – polymorphism information content. Values calculated based on sequence information are represented by the superscript S while the ones based on length information by L. The last seven markers separated from the remaining ones correspond to the markers excluded for the complete analyses either by excess of missing data or lack of variation.

Marker	All samples									*E. europaeus*									*E. roumanicus*								
	% missing	Na^S	Na^L	H_O^S	H_O^L	H_E^S	H_E^L	PIC^S	PIC^L	% missing	Na^S	Na^L	H_O^S	H_O^L	H_E^S	H_E^L	PIC^S	PIC^L	% missing	Na^S	Na^L	H_O^S	H_O^L	H_E^S	H_E^L	PIC^S	PIC^L
E1	31,71	21	19	0,79	0,77	0,91	0,91	0,89	0,89	43,90	13	13	0,91	0,91	0,79	0,79	0,85	0,75	19,51	14	12	0,70	0,67	0,88	0,87	0,75	0,84
E10	46,34	12	10	0,61	0,55	0,85	0,82	0,83	0,79	85,37	6	6	0,17	0,17	0,86	0,86	0,79	0,76	7,32	9	7	0,68	0,61	0,82	0,78	0,76	0,75
E11	9,76	22	12	0,68	0,61	0,92	0,86	0,91	0,84	17,07	13	10	0,68	0,65	0,86	0,84	0,84	0,80	2,44	15	8	0,68	0,58	0,87	0,77	0,83	0,72
E13	0,00	37	12	0,76	0,68	0,94	0,88	0,93	0,86	0,00	26	10	0,88	0,73	0,94	0,89	0,82	0,87	0,00	18	12	0,63	0,63	0,85	0,84	0,93	0,80
E16	1,22	16	11	0,70	0,68	0,90	0,86	0,88	0,84	2,44	10	9	0,75	0,75	0,86	0,86	0,86	0,83	0,00	13	8	0,66	0,61	0,88	0,83	0,83	0,79
E18	9,76	8	6	0,41	0,24	0,78	0,65	0,74	0,59	17,07	4	3	0,38	0,03	0,54	0,06	0,60	0,06	2,44	7	6	0,43	0,43	0,64	0,63	0,42	0,59
E19	2,44	12	7	0,33	0,20	0,69	0,62	0,66	0,56	2,44	3	2	0,03	0,00	0,07	0,05	0,79	0,05	2,44	12	7	0,63	0,40	0,82	0,67	0,07	0,61
E2	8,54	23	12	0,63	0,60	0,87	0,82	0,85	0,79	17,07	17	10	0,71	0,65	0,88	0,84	0,63	0,80	0,00	11	7	0,56	0,56	0,67	0,63	0,86	0,58
E20	32,93	19	18	0,49	0,49	0,92	0,92	0,91	0,91	39,02	12	12	0,48	0,48	0,88	0,88	0,80	0,85	26,83	11	11	0,50	0,50	0,84	0,84	0,85	0,80
E22	0,00	15	11	0,51	0,35	0,83	0,71	0,81	0,68	0,00	7	5	0,39	0,10	0,54	0,12	0,82	0,12	0,00	13	10	0,63	0,61	0,84	0,84	0,47	0,81
E23	10,98	49	15	0,99	0,97	0,94	0,85	0,93	0,83	19,51	28	13	0,97	0,97	0,94	0,87	0,85	0,84	2,44	25	6	1,00	0,98	0,87	0,64	0,92	0,58
E26	30,49	8	6	0,37	0,16	0,76	0,60	0,71	0,54	41,46	6	5	0,33	0,33	0,61	0,61	0,43	0,55	19,51	4	3	0,39	0,03	0,54	0,09	0,56	0,09
E27	0,00	7	4	0,12	0,09	0,57	0,56	0,48	0,46	0,00	6	3	0,15	0,07	0,37	0,31	0,24	0,27	0,00	3	3	0,10	0,10	0,26	0,26	0,33	0,24
E28	1,22	7	4	0,27	0,11	0,72	0,60	0,67	0,51	2,44	7	4	0,25	0,18	0,49	0,40	0,40	0,35	0,00	5	3	0,29	0,05	0,47	0,12	0,44	0,11
E29	23,17	11	3	0,37	0,21	0,83	0,47	0,81	0,37	21,95	6	2	0,38	0,13	0,66	0,31	0,71	0,26	24,39	8	3	0,35	0,29	0,75	0,54	0,61	0,42
E3	37,80	23	14	0,45	0,41	0,89	0,87	0,87	0,85	17,07	10	8	0,50	0,50	0,77	0,77	0,90	0,72	58,54	15	9	0,35	0,24	0,94	0,86	0,73	0,82
E30	0,00	8	5	0,46	0,33	0,78	0,69	0,75	0,64	0,00	5	4	0,34	0,10	0,58	0,27	0,65	0,25	0,00	6	4	0,59	0,56	0,71	0,69	0,51	0,61
E31	9,76	5	3	0,30	0,26	0,66	0,64	0,60	0,57	17,07	3	3	0,06	0,06	0,09	0,09	0,53	0,08	2,44	5	3	0,50	0,43	0,61	0,55	0,08	0,44
E34	0,00	12	8	0,51	0,40	0,84	0,77	0,82	0,73	0,00	9	7	0,39	0,20	0,65	0,39	0,77	0,37	0,00	11	8	0,63	0,61	0,80	0,79	0,62	0,75
E35	21,95	5	5	0,13	0,13	0,61	0,61	0,55	0,55	29,27	5	5	0,28	0,28	0,61	0,61	0,11	0,56	14,63	3	3	0,00	0,00	0,11	0,11	0,56	0,11
E36	9,76	35	13	0,78	0,62	0,95	0,90	0,94	0,88	17,07	23	12	0,82	0,56	0,92	0,85	0,87	0,82	2,44	19	10	0,75	0,68	0,89	0,87	0,90	0,84
E37	0,00	7	5	0,33	0,29	0,73	0,70	0,69	0,65	0,00	5	4	0,12	0,07	0,35	0,27	0,67	0,25	0,00	7	5	0,54	0,51	0,73	0,72	0,33	0,65
E4	10,98	9	4	0,34	0,26	0,69	0,64	0,62	0,56	17,07	6	3	0,18	0,03	0,27	0,09	0,47	0,08	4,88	6	4	0,49	0,46	0,55	0,54	0,26	0,44
E5	47,56	16	15	0,21	0,21	0,70	0,65	0,68	0,63	34,15	6	5	0,11	0,11	0,39	0,27	0,86	0,26	60,98	13	13	0,38	0,38	0,90	0,90	0,37	0,86

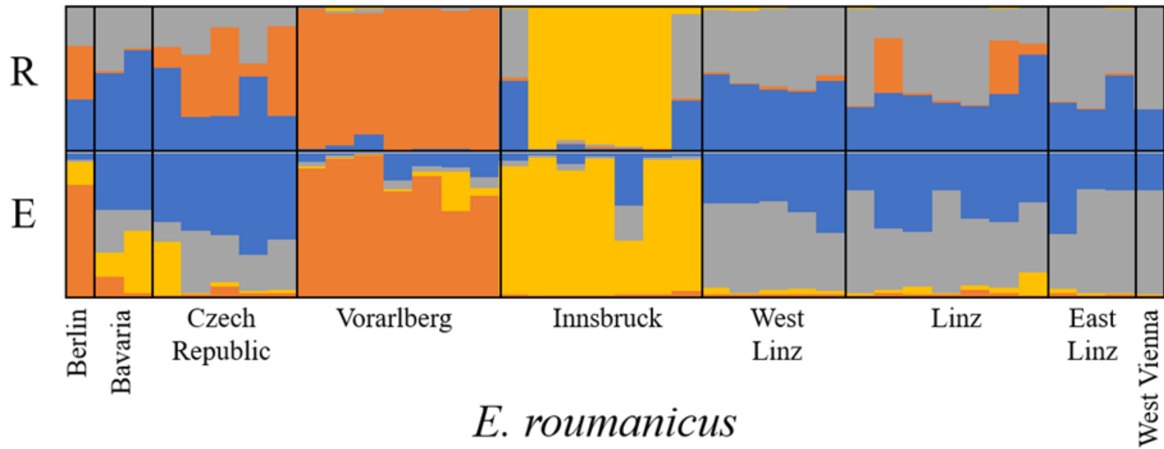
E8	34,15	7	4	0,24	0,17	0,69	0,57	0,66	0,49	9,76	3	2	0,30	0,19	0,50	0,39	0,66	0,31	58,54	5	4	0,12	0,12	0,72	0,70	0,43	0,64
E9	19,51	11	7	0,52	0,42	0,81	0,58	0,78	0,54	17,07	9	6	0,94	0,79	0,70	0,59	0,54	0,54	21,95	6	4	0,06	0,03	0,59	0,53	0,66	0,47
W10	1,22	10	8	0,38	0,38	0,72	0,66	0,68	0,62	2,44	7	6	0,65	0,65	0,78	0,77	0,18	0,72	0,00	6	4	0,12	0,12	0,19	0,16	0,73	0,16
W11	29,27	23	10	0,53	0,48	0,93	0,88	0,91	0,86	19,51	20	8	0,73	0,64	0,94	0,83	0,71	0,80	39,02	7	6	0,28	0,28	0,76	0,72	0,92	0,65
W12	2,44	50	22	0,79	0,75	0,97	0,93	0,96	0,92	0,00	20	14	0,80	0,78	0,94	0,89	0,94	0,87	4,88	34	12	0,77	0,72	0,96	0,84	0,92	0,81
W14	1,22	15	8	0,63	0,62	0,88	0,77	0,87	0,73	0,00	10	7	0,56	0,54	0,80	0,77	0,71	0,73	2,44	7	6	0,70	0,70	0,75	0,73	0,76	0,68
W16	19,51	7	4	0,41	0,21	0,49	0,25	0,46	0,23	12,20	5	3	0,42	0,31	0,41	0,31	0,45	0,29	26,83	4	3	0,40	0,10	0,54	0,16	0,38	0,15
W19	19,51	23	15	0,64	0,62	0,92	0,89	0,90	0,88	7,32	13	9	0,55	0,53	0,88	0,85	0,85	0,82	31,71	16	13	0,75	0,75	0,88	0,85	0,86	0,82
W21	40,24	4	2	0,12	0,12	0,61	0,35	0,54	0,29	51,22	3	2	0,10	0,10	0,56	0,51	0,16	0,37	29,27	4	2	0,14	0,14	0,16	0,13	0,44	0,12
W23	1,22	11	8	0,59	0,58	0,84	0,83	0,81	0,80	0,00	7	6	0,68	0,66	0,74	0,73	0,60	0,67	2,44	7	7	0,50	0,50	0,66	0,66	0,68	0,60
W29	13,41	14	9	0,61	0,58	0,87	0,80	0,85	0,77	7,32	10	9	0,71	0,71	0,84	0,84	0,59	0,80	19,51	7	5	0,48	0,42	0,66	0,63	0,81	0,55
W30	14,63	30	23	0,69	0,67	0,93	0,92	0,92	0,91	0,00	17	11	0,66	0,63	0,86	0,82	0,86	0,80	29,27	16	15	0,72	0,72	0,88	0,88	0,85	0,85
W31	0,00	33	19	1,00	1,00	0,93	0,90	0,92	0,89	0,00	23	15	1,00	1,00	0,89	0,85	0,80	0,83	0,00	14	10	1,00	1,00	0,83	0,78	0,87	0,75
W32	45,12	9	9	0,42	0,42	0,57	0,57	0,54	0,54	4,88	5	5	0,41	0,41	0,46	0,46	0,72	0,42	85,37	6	6	0,50	0,50	0,82	0,82	0,42	0,72
W5	23,17	13	8	0,60	0,46	0,88	0,78	0,86	0,75	31,71	8	5	0,46	0,46	0,80	0,71	0,71	0,64	14,63	9	6	0,71	0,46	0,76	0,54	0,75	0,48
W7	1,22	16	9	0,69	0,69	0,90	0,84	0,89	0,82	0,00	11	9	0,73	0,73	0,83	0,82	0,77	0,78	2,44	10	7	0,65	0,65	0,80	0,80	0,79	0,76
W8	0,00	29	23	0,85	0,84	0,95	0,94	0,94	0,93	0,00	23	17	0,80	0,78	0,92	0,88	0,91	0,86	0,00	16	16	0,90	0,90	0,92	0,92	0,90	0,91
W9	37,80	15	9	0,45	0,43	0,89	0,85	0,87	0,82	14,63	9	7	0,57	0,54	0,82	0,79	0,76	0,75	60,98	9	7	0,19	0,19	0,81	0,80	0,78	0,75
E24	1,22	1	1	0,00	0,00	0,00	0,00	0,00	0,00	2,44	1	1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1	1	0,00	0,00	0,00	0,00	0,00	0,00
E7	54,88	9	6	0,11	0,11	0,74	0,59	0,70	0,50	65,85	6	5	0,29	0,29	0,76	0,52	0,41	0,47	43,90	5	3	0,00	0,00	0,44	0,41	0,69	0,35
W1	53,66	11	6	0,21	0,21	0,82	0,77	0,78	0,72	26,83	7	6	0,23	0,23	0,72	0,71	0,65	0,64	80,49	4	3	0,13	0,13	0,74	0,69	0,67	0,58
W13	67,07	5	5	0,22	0,22	0,60	0,60	0,55	0,55	92,68	2	2	0,00	0,00	0,53	0,53	0,54	0,35	41,46	4	4	0,25	0,25	0,60	0,60	0,35	0,54
W15	62,20	5	4	0,00	0,00	0,39	0,39	0,37	0,36	80,49	5	4	0,00	0,00	0,80	0,73	0,00	0,63	43,90	1	1	0,00	0,00	0,00	0,00	0,71	0,00
W18	58,54	4	3	0,18	0,00	0,54	0,39	0,49	0,34	82,93	2	2	0,00	0,00	0,44	0,44	0,28	0,33	34,15	3	2	0,22	0,00	0,32	0,07	0,33	0,07
W33	57,32	10	7	0,43	0,40	0,63	0,56	0,61	0,53	19,51	9	6	0,42	0,39	0,58	0,53	0,56	0,49	95,12	3	3	0,50	0,50	0,83	0,83	0,56	0,56

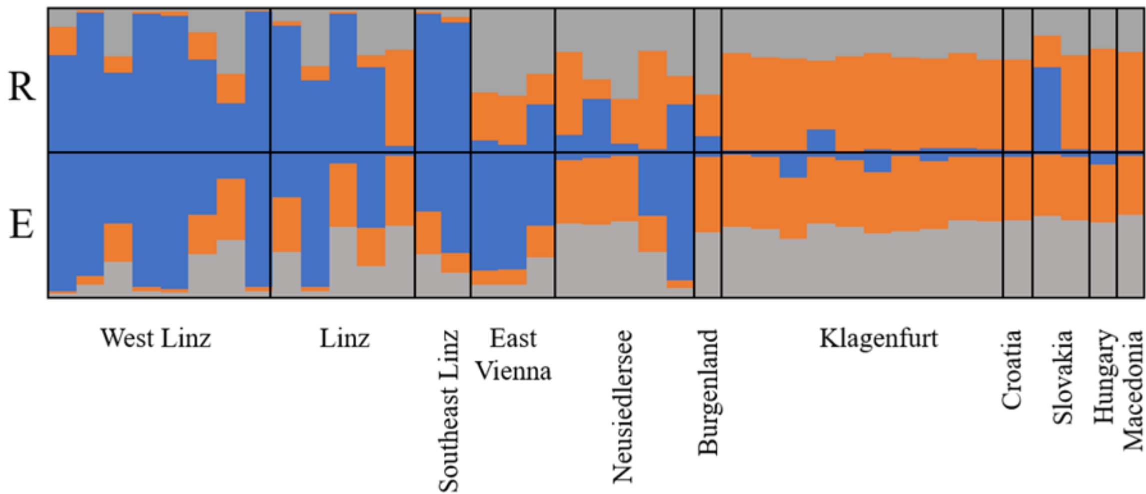


Supplementary Figure 2.1: Correlation between geographical and genetic distance among individuals. Distances was calculated among individuals because of lack of population sampling. Upper graph includes individuals from *E. europaeus* only while the bottom one contains individuals from *E roumanicus*. errata Error: *E. europeaus* Correction: *E. europaeus*



Supplementary Figure 2.2: Structure analyses for all three datasets (all samples, only *E. europaeus,* only *E. roumanicus*) considering all markers and alleles called based on both sequence and length information. Only the results for the optimum K values are shown: K=2 for all samples; K=3 for *E. europaeus*; and K=5 for *E. roumanicus.*





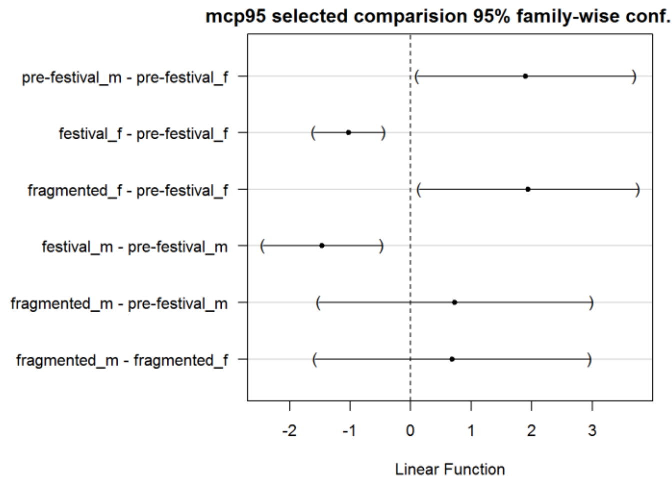
Supplementary Figure 2.3: Structure analyses for all three datasets (all samples, only E. europaeus, only E. roumanicus) considering markers developed specifically for *E. roumanicus* (R) and *E. europaeus* (E) and alleles called based on sequence information. Only the results for the optimum K values are shown: K=4 for E. europaeus; K=3 for E. europaeus

4 Supplementary information Chapter 4: Distinguishing spatial from temporal effects in disturbance biology: Hedgehogs in the urban matrix of habitat fragmentation and noise pollution

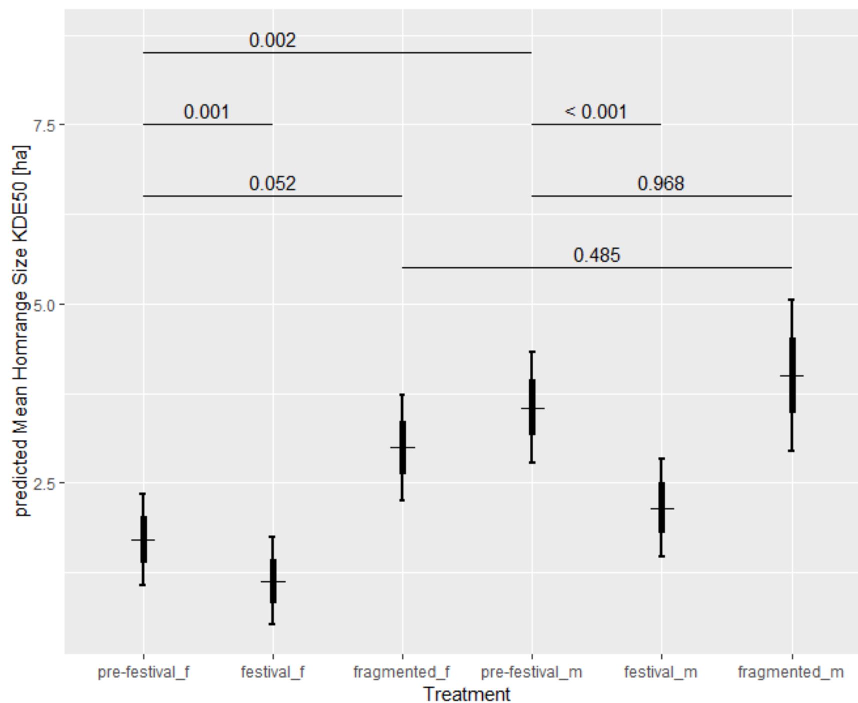
Supplementary Table 4.1: Linear model, outcome of ANOVA and results of the multiple comparisons of MCP95

```
lme_mcp <- lme(mcp95_area ~ treatment*sex, random = ~ 1|animal_id, res.new,
  weights = varPower())
Anova(lme_mcp)
## Analysis of Deviance Table (Type II tests)
##
## Response: mcp95_area
##   Chisq Df Pr(>Chisq)
## treatment 54.8210 2 1.247e-12 ***
## sex  6.4797 1 0.01091 *
## treatment:sex 1.7432 2 0.41828
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(mod_mcp_plot, linfct = mcp(inter_Treat_Sex = K)))
## Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
## Fit: lme.formula(fixed = mcp95_area ~ inter_Treat_Sex, data = res.new,
## random = ~1 | animal_id, weights = varPower())
##
## Linear Hypotheses:
##  Estimate Std. Error z value Pr(>|z|)
## pre-festival_m - pre-festival_f == 0 1.8968 0.6891 2.753 0.0327*
## festival_f - pre-festival_f == 0 -1.0290 0.2214 -4.648 <0.001***
## fragmented_f - pre-festival_f == 0 1.9421 0.6945 2.796 0.0291*
## festival_m - pre-festival_m == 0 -1.4665 0.3770 -3.890 <0.001***
## fragmented_m - pre-festival_m == 0 0.7302 0.8663 0.843 0.9200
## fragmented_m - fragmented_f == 0 0.6849 0.8706 0.787 0.9389
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

Supplementary information Chapter 4: Distinguishing spatial from temporal effects in disturbance biology: Hedgehogs in the urban matrix of habitat fragmentation and noise pollution



Supplementary Figure 4.1: Multiple Comparisons of Means for the MCP95



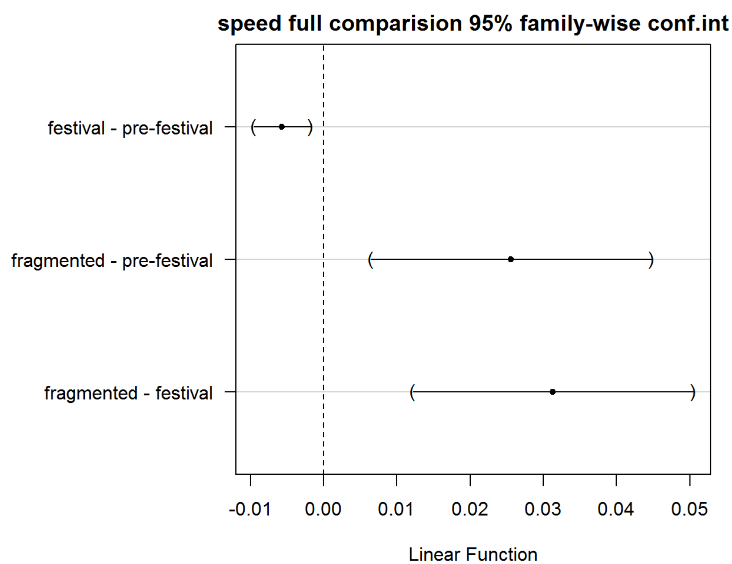
Supplementary Figure 4.2: Results of the KDE50

Supplementary Table 4.2: Linear model, outcome of ANOVA and results of the multiple comparisons of KDE50

```
mod_kde <- lme(kde50_area ~ treatment*sex, random = ~ 1|animal_id, res.new,
 weights = varPower())
car::Anova(mod_kde)
## Analysis of Deviance Table (Type II tests)
##
## Response: kde50_area
##  Chisq Df Pr(>Chisq)
## treatment 44.9871 2 1.703e-10 ***
## sex 10.2643 1 0.001356 **
## treatment:sex 5.6387 2 0.059644 .
```

Supplementary Table 4.3: Linear model, outcome of ANOVA and multiple comparisons for speed

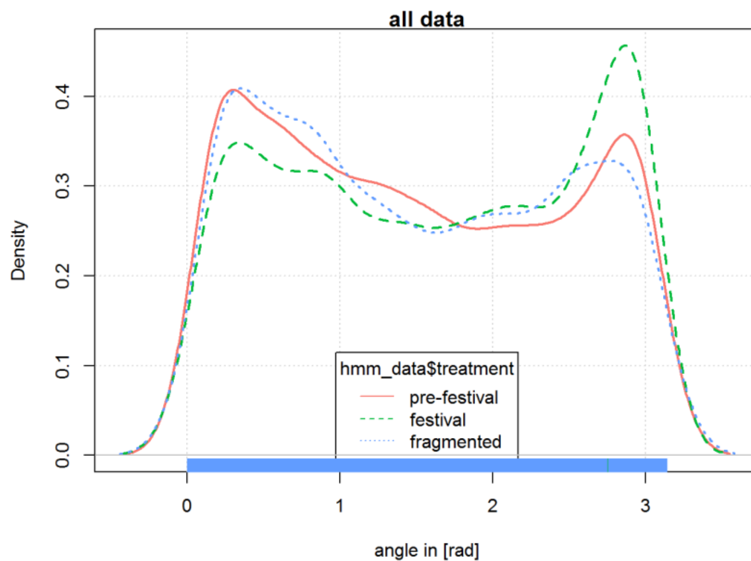
```
mod_speed <- lmer(sqrt(speed) ~ treatment*sex + (1 |animal_id), mydata.sf.EOBS)

Anova(mod_speed)

mod_speed_res <- multcomp::glht(mod_speed, linfct = multcomp::mcp(treatment = "Tukey"),
interaction_average = FALSE, covariate_average = TRUE)

Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: Tukey Contrasts
##
##
## Fit: lmer(formula = sqrt(speed) ~ treatment * sex + (1 | animal_id),
## data = mydata.sf.EOBS)
##
## Linear Hypotheses:
##     Estimate Std. Error z value Pr(>|z|)
## festival - pre-festival == 0 -0.005701 0.001715 -3.325 0.00206 **
## fragmented - pre-festival == 0 0.025613 0.008429 3.038 0.00511 **
## fragmented - festival == 0 0.031314 0.008425 3.717 < 0.001 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```



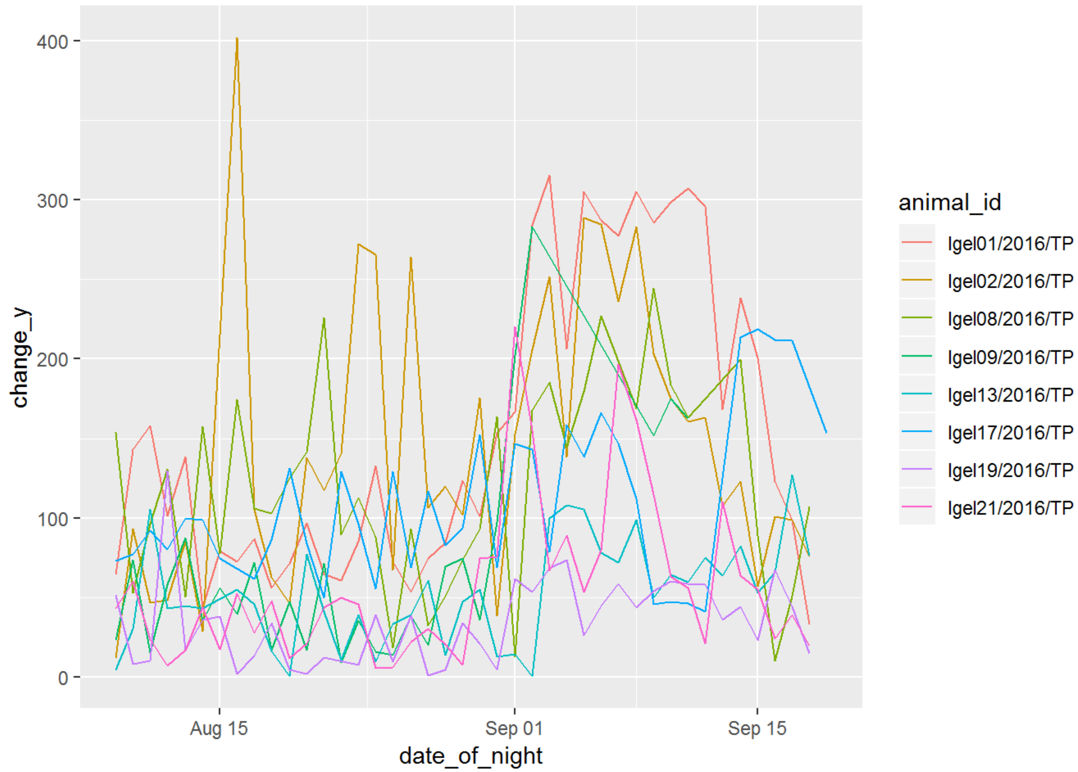
Supplementary Figure 4.3: speed pairwise comparison of treatments

Supplementary Table 4.4: Loop for permutation: example for the comparison of the pre-festival and festival data using the ks.test

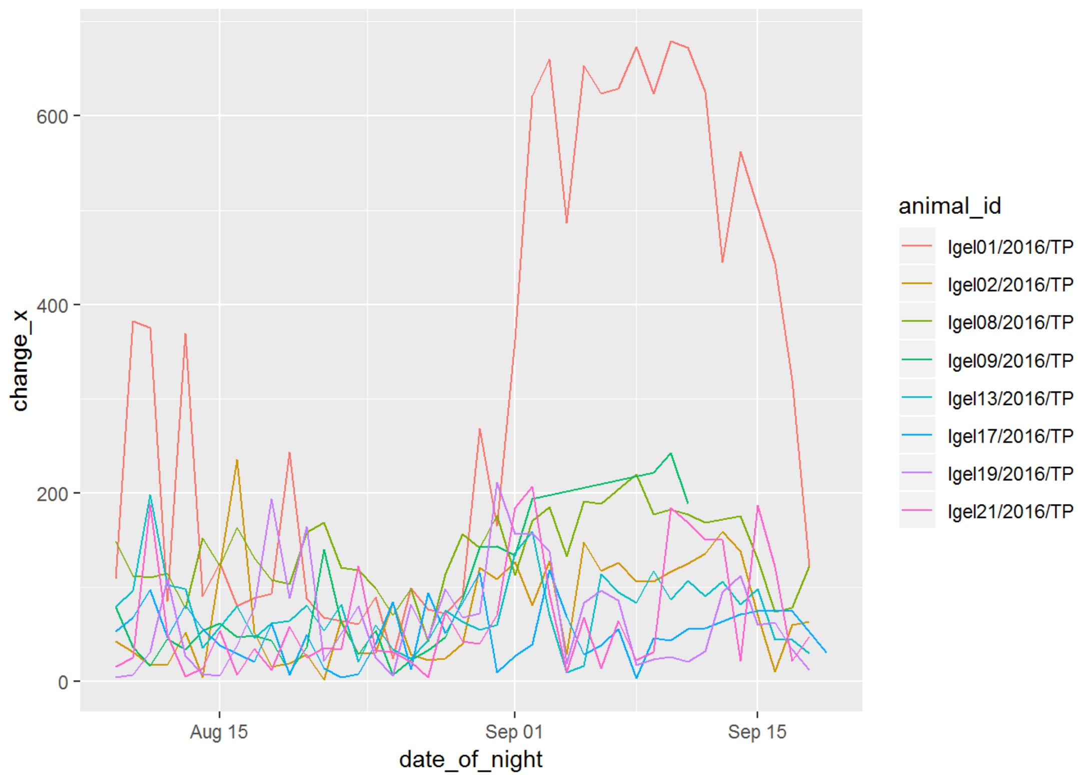
```
loop for pre-festival vs. festival
n = 1000
result <- data.frame(p_value = rep(NA,n), valueW = NA)
for (i in 1:n){
 hmm.new <- hmm_data %>%
 group_by(ID) %>%
 sample_n(170)

result.test <- ks.test(abs(hmm.new$angle[hmm.new$treatment== "pre-festival"])
,abs(hmm.new$angle[hmm.new$treatment== "festival"]))

result$p_value[i] <- result.test$p.value
result$valueW[i] <- result.test$statistic
}
result_pre_fest <- result
mean(result_pre_fest$p_value)
0.1138248
```



Supplementary Figure 4.4: all turning Angels of all hedghogs

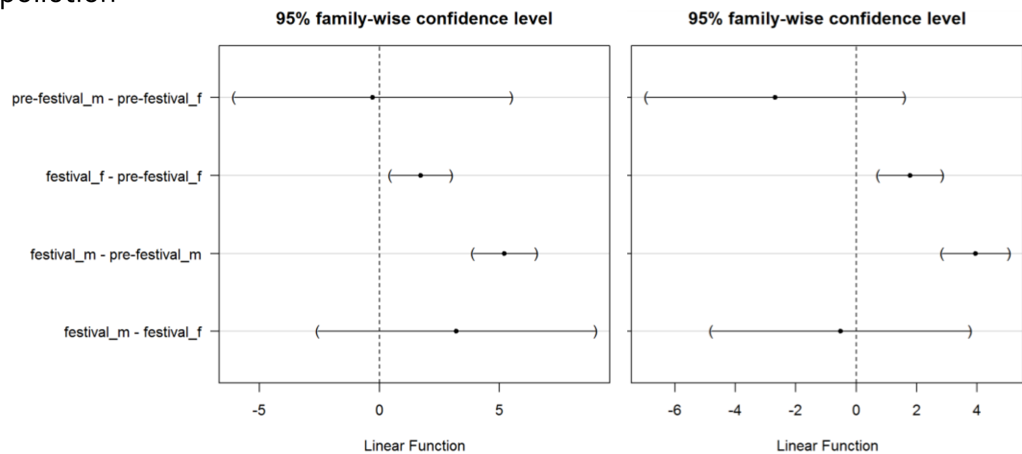


Supplementary Figure 4.5: Raw data latitude of the centroid values per day over time construction work started at the 28th of August and ended on the 16th September



Supplementary Figure 4.6: Rawdata longitude of the centroid values per day over time construction work started at the 28th of August and ended on the 16th September

Supplementary information Chapter 4: Distinguishing spatial from temporal effects in disturbance biology: Hedgehogs in the urban matrix of habitat fragmentation and noise pollution



Supplementary Figure 4.7: left longitudinal data, right: latidudinal data

Supplementary Table 4.5: Linear model and outcome of ANOVA of longitudinal data and latitudinal

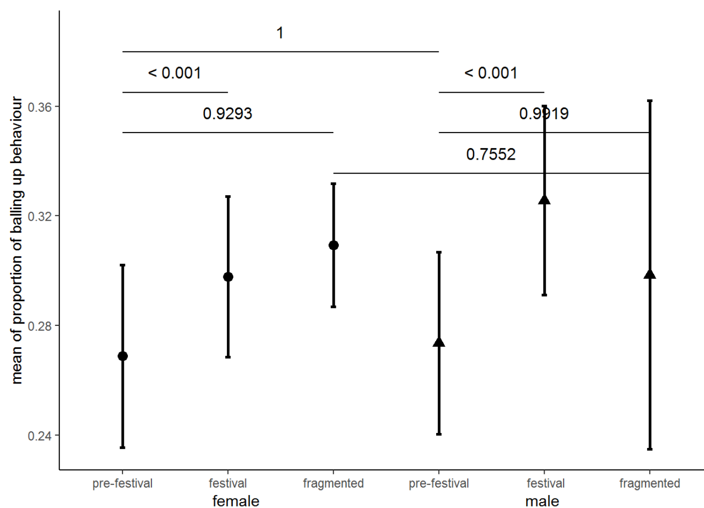
```
mod_nom_x <- lmer(sqrt(change_x) ~ treatment*sex + (1 |animal_id), res.fest)
car::Anova(mod_nom_x)
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(change_x)
## Chisq Df Pr(>Chisq)
## treatment 80.5897 1 < 2.2e-16 ***
## sex 0.3572 1 0.55
## treatment:sex 21.4375 1 3.655e-06 ***
```

```
mod_nom_y <- lmer(sqrt(change_y) ~ treatment*sex + (1 |animal_id), res.fest)

car::Anova(mod_nom_y)
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: sqrt(change_y)
## Chisq Df Pr(>Chisq)
## treatment 80.858 1 < 2.2e-16 ***
## sex 0.886 1 0.3465542
## treatment:sex 11.790 1 0.0005954 ***
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Supplementary Table 4.6: Linear model, outcome of ANOVA and results of the multiple comparisons for balled up behaviour

```
car::Anova(glmer(cbind(bu, all - bu) ~ treatment*sex + (1 | hedgehog.id),
  data = daily.data,
  family = binomial))

Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: cbind(bu, all - bu)
##   Chisq Df Pr(>Chisq)
## treatment 598.4354 2 < 2.2e-16 ***
## sex  0.4289 1 0.512551
## treatment:sex 11.8406 2 0.002684 **
```

```
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(gm1, linfct = mcp(inter_Treat_Sex = K)))
##
## Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
## Fit: glmer(formula = cbind(bu, all - bu) ~ inter_Treat_Sex + (1 |
## hedgehog.id), data = daily.data, family = binomial)
##
## Linear Hypotheses:
##     Estimate Std. Error z value Pr(>|z|)
## pre-festival_m - pre-festival_f == 0 0.03941 0.25135 0.157 1.000
## festival_f - pre-festival_f == 0 0.15268 0.01015 15.040 <1e-04***
## festival_m - pre-festival_m == 0 0.20006 0.01022 19.571 <1e-04 ***
## fragmented_f - pre-festival_f == 0 0.20941 0.25133 0.833 0.929
## fragmented_m - pre-festival_m == 0 -0.12687 0.25162 -0.504 0.992
## fragmented_m - fragmented_f == 0 -0.29687 0.25160 -1.180 0.755
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```

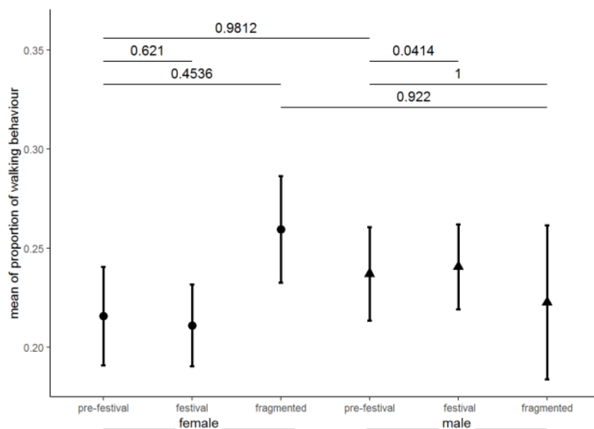


Supplementary Figure 4.8: mean proportion of balling up behaviour against treatments, whiskers indicate confidence interval

Supplementary Table 4.7: Linear model, outcome of ANOVA and results of the multiple comparisons of walking behaviour

```
car::Anova(glmer(cbind(w, all - w) ~ treatment*sex + (1 | hedgehog.id),
  data = daily.data,
  family = binomial))
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: cbind(w, all - w)
##  Chisq Df Pr(>Chisq)
```

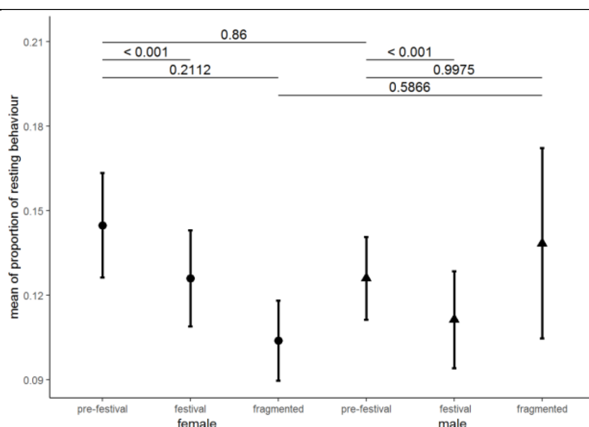
```
## treatment 9.5608 2 0.008393 **
## sex  0.0205 1 0.886235
## treatment:sex 1.9247 2 0.381989
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

##
## Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
## Fit: glmer(formula = cbind(w, all - w) ~ inter_Treat_Sex + (1 | hedgehog.id),
## data = daily.data, family = binomial)
##
## Linear Hypotheses:
##     Estimate Std. Error z value Pr(>|z|)
## pre-festival_m - pre-festival_f == 0 0.10452 0.17186 0.608 0.9812
## festival_f - pre-festival_f == 0 0.01557 0.01133 1.374 0.6211
## festival_m - pre-festival_m == 0 0.02970 0.01110 2.675 0.0419 *
## fragmented_f - pre-festival_f == 0 0.27632 0.17184 1.608 0.4536
## fragmented_m - pre-festival_m == 0 0.02479 0.17218 0.144 1.0000
## fragmented_m - fragmented_f == 0 -0.14701 0.17215 -0.854 0.9220
##
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```



Supplementary Figure 4.9: mean proportion of walking behaviour against treatments, whiskers indicate confidence interval

Supplementary Table 4.8: Linear model, outcome of ANOVA and results of the multiple comparisons of resting behaviour

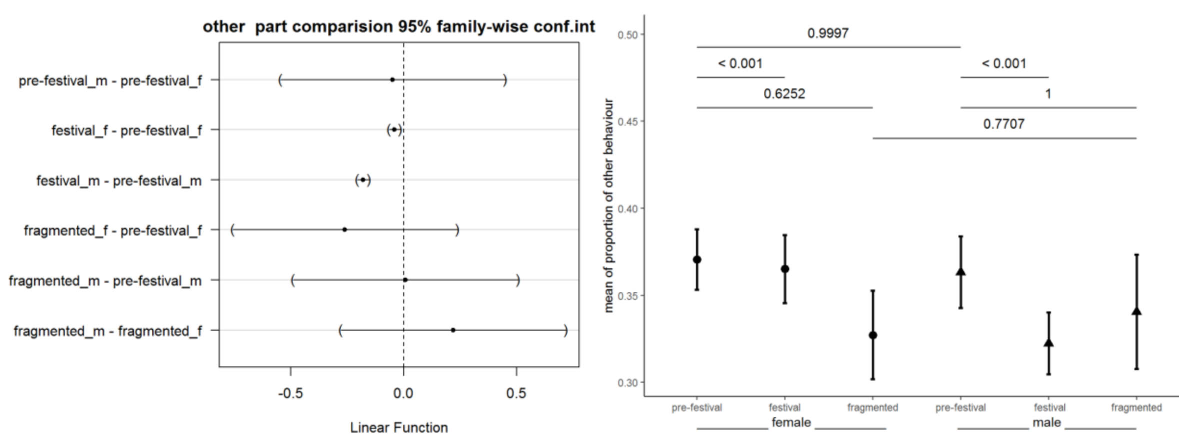
```
## Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: cbind(ar, all - ar)
##   Chisq Df Pr(>Chisq)
## treatment 203.2121 2 < 2.2e-16 ***
## sex  0.2557 1 0.6131
## treatment:sex 54.6765 2 1.34e-12 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
##
## Fit: glmer(formula = cbind(ar, all - ar) ~ inter_Treat_Sex + (1 |
## hedgehog.id), data = daily.data, family = binomial)
##
## Linear Hypotheses:
##     Estimate Std. Error z value Pr(>|z|)
## pre-festival_m - pre-festival_f == 0 -0.22498 0.22531 -0.999 0.860
## festival_f - pre-festival_f == 0 -0.20539 0.01335 -15.391 <0.001 ***
## festival_m - pre-festival_m == 0 -0.06227 0.01458 -4.269 <0.001 ***
## fragmented_f - pre-festival_f == 0 -0.45706 0.22532 -2.028 0.211
## fragmented_m - pre-festival_m == 0 0.08880 0.22570 0.393 0.997
## fragmented_m - fragmented_f == 0 0.32088 0.22571 1.422 0.587
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```



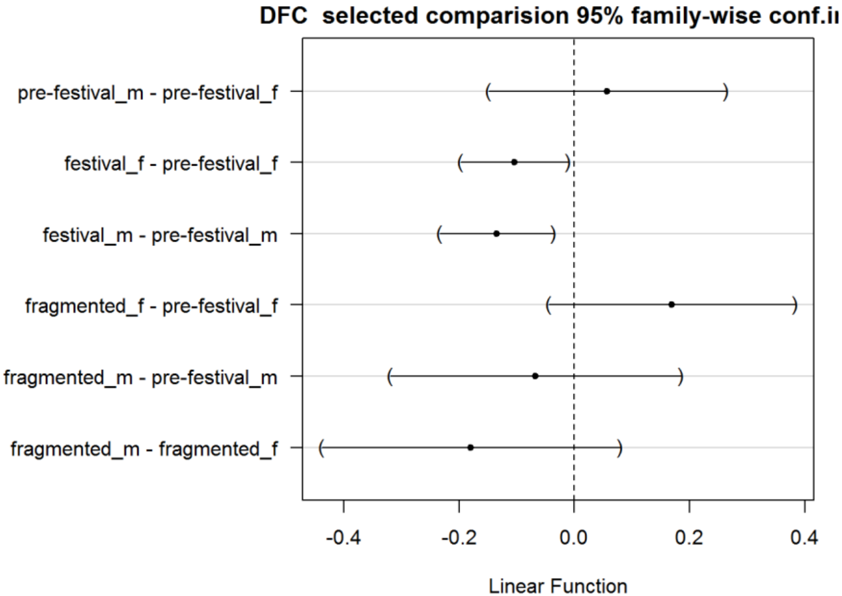
Supplementary Figure 4.10: mean proportion of behaviour identified as resting against treatment

Supplementary Table 4.9: Linear model, outcome of ANOVA and results of the multiple comparisons of other (not identified) behaviour

```
# Analysis of Deviance Table (Type II Wald chisquare tests)
##
## Response: cbind(other, all - other)
##   Chisq Df Pr(>Chisq)
## treatment 248.2961 2 <2e-16 ***
## sex  0.1481 1 0.7004
## treatment:sex 105.6829 2 <2e-16 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Simultaneous Tests for General Linear Hypotheses
##
## Multiple Comparisons of Means: User-defined Contrasts
##
## Fit: glmer(formula = cbind(other, all - other) ~ inter_Treat_Sex +
## (1 | hedgehog.id), data = daily.data, family = binomial)
##
## Linear Hypotheses:
## Estimate Std. Error z value Pr(>|z|)
## pre-festival_m - pre-festival_f == 0 -0.048830 0.190356 -0.257 0.999679
## festival_f - pre-festival_f == 0 -0.040495 0.009461 -4.280 0.000107 ***
## festival_m - pre-festival_m == 0 -0.179777 0.009839 -18.272 < 1e-04 ***
## fragmented_f - pre-festival_f == 0 -0.260492 0.190355 -1.368 0.625203
## fragmented_m - pre-festival_m == 0 0.008588 0.190594 0.045 1.000000
## fragmented_m - fragmented_f == 0 0.220250 0.190591 1.156 0.770731
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
## (Adjusted p values reported -- single-step method)
```



Supplementary Figure 4.11: left: estimates in pairwise comparisons; whiskers indicate 95% confidence interval right mean proportion of behaviour not classified other, whiskers indicate confidence interval

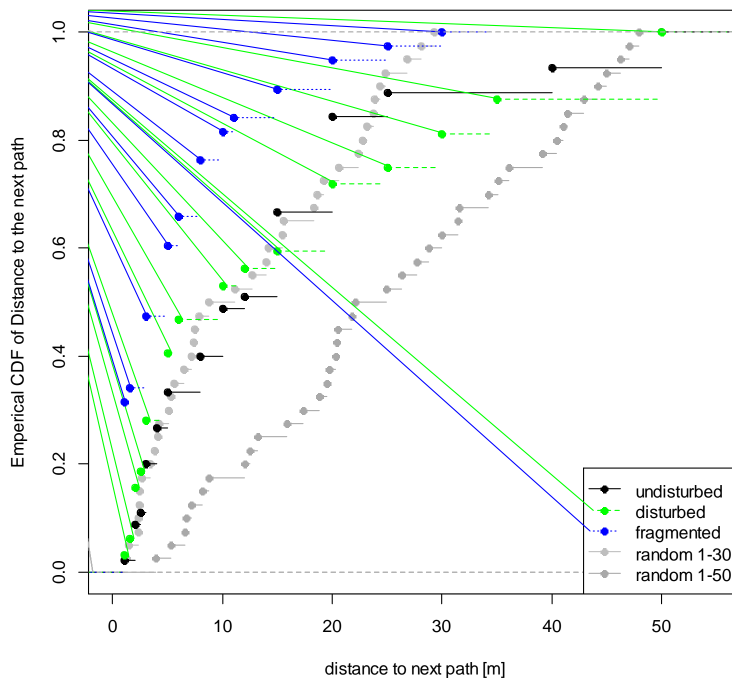


Supplementary Figure 4.12: Estimates of Degree of Functional Coupling pairwise comparison

Supplementary Table 4.10: Nesting details

Sex	**Hedgehog ID**	**Tracking period (days)**	**Total No. of nests**	**Total No. of changes**	**Days /nest**	**Days/ change**	**Days normal**	**Days festival**	**No. of nests normal**	**No. of nests festival**	**Days/ nest normal**	**Days/ nest festival**	**No. of changes normal**	**No. of changes festival**	**Days/ change normal**	**Days/ change festival**
Males	1	41	9	8	4.55	5.1	23	18	5	6	4.6	3.0	3	5	7.7	3.6
	5	19	5	5	3.8	3.8	19	0	5	0	3.8		4	1	4.8	0
	9	42	10	20	4.2	2.1	24	18	6	5	4.0	3.6	14	6	1.7	3.0
	11	33	5	18	6.6	1.8	18	14	4	4	4.5	3.5	7	11	2.6	1.3
	14	42	8	19	5.25	2.2	24	18	5	5	4.8	3.6	9	10	2.7	1.8
	18	42	5	4	8.4	10.5	24	18	3	3	8	6.0	0	4	23.0	4.5
	19	42	9	14	4.66	3.0	24	18	7	4	3.4	4.5	10	4	2.4	4.5
	21	42	8	23	5.25	1.8	24	18	5	7	4.8	2.6	8	15	3.0	1.2
	22	35	9	22	3.88	1.6	17	18	6	7	2.8	2.6	11	11	1.5	1.6
			7.6	**14.8**	**5.2**	**3.6**			**5.1**	**4.6**	**4.5**	**3.7**	**7.3**	**7.4**	**5.5**	**2.4**
Females	2	42	4	11	10.5	3.8	24	18	4	2	6.0	9.0	6	5	4.0	3.6
	7	42	2	5	21	8.4	24	18	2	1	12.0	18.0	5	0	4.8	19.0
	8	41	6	8	6.83	5.1	23	18	2	5	11.5	3.6	0	8	22.0	2.3
	13	42	9	15	4.66	2.8	24	18	7	5	3.4	3.6	9	6	2.7	3.0
	16	42	6	14	7	3.0	24	18	6	3	4.0	6.0	9	5	2.7	3.6
	17	42	6	9	7	4.7	24	18	5	3	4.8	6.0	6	3	4.0	6.0
	20	39	4	3	9.75	13.0	21	18	4	1	5.3	18.0	3	0	7.0	19.0
	24	23	2	3	11.5	7.7	6	17	1	2	6.0	8.5	0	3	5.00	5.7
			4.9	**8.5**	**9.8**	**6.1**			**3.9**	**2.8**	**6.6**	**9.1**	**4.8**	**3.8**	**6.5**	**7.8**

Traits um die Fragmentierung der Parks darzustellen



Supplementary Figure 4.13: Empirical Cumulative Distribution Function of Distance to the next path, indicating the fragmentation of the habitat

There was no differnces between sexes in the distance to path so the following comparison are for the whole datasets. There was no differences between the undisturbed and disturbed data. But all three differ significantly from a random number: showing the near association of hedgehogs in the fragmented habitat nearer to path.

Supplementary Table 4.11: Nest protocol

Date	Time	Recorder
GPS-Nest		Nest-ID
Hedgehog-ID	Transmission frequency	Type of nest (1-6) 1) In hollow tree or stump 2) under human left-overs (garbage/plastic) 3) in burrow 4) under a thin layer of leaves, 5) supported by branches/twigs or deadwood 6) thick layer of leaves → multiple choice possible
Shrub-density light/medium/ denselight = it is possible to walk through the shrub without touching the plants, unrestricted view; medium = you touch some plants by passing through the shrub, light restricted view; dense = you cannot walk through the shrub without touching, restricted view	Distance meadow [m] shortest distance to closest meadow	Distance path/road [m] shortest distance to closest path/road
Nest-diameter	Nest-height	Direction entrance/exit hold compass over nest entrance/exit and determine degree (0° - 360°)
Nest-material	Plants at the nest Plants that touch/support/are a part of the nest Groundcover yes / no The vegetation, which covers the area (ground) around the nest, is used and noticed as allocated type of vegetation. E.g. Ivy or yellow archangel	Plants around the nest (5m) maple, beech, hornbeam, horse-chestnut, poplar, oak, elm, robinia, lime, rhus typhina, black walnut, ash, yew, plane, willow, elder, mock orange bush, blackthorn, honeysuckle, whitethorn, clematis, common hazel, privet, ilex, rosa canina, currant, blackberry/dewberry, rubus odoratus, snowberry, dogwood, spindle tree, evening primrose, goldenrod, celandine, nettle, yellow archangel, ivy, wild hop
Anomalies e.g. nest open; hedgehog visible; special structure; unusual surrounding etc.		

Supplementary Table 4.12: Nest survival raw data Treptower Park 2016

sex	individual	nest	nesttime	censor	coding	research_interval	disturbance
m	1	a	5	yes	0	before	no
m	1	b	2	no	1	before	no
m	1	c	11	yes	0	before	no
m	1	c	3	no	1	during	disturb
m	1	d	6	no	1	during	disturb
m	1	e	1	no	1	during	disturb
m	1	f	2	no	1	during	disturb
m	1	g	1	no	1	during	disturb
m	1	h	5	yes	0	during	disturb
m	1	h	1	no	1	after	no
m	1	i	4	yes	0	after	no
f	2	a	5	yes	0	before	no
f	2	b	2	no	1	before	no
f	2	a	2	no	1	before	no
f	2	b	1	no	1	before	no
f	2	a	1	no	1	before	no
f	2	b	1	no	1	before	no
f	2	c	7	yes	0	before	no
f	2	c	8	no	1	during	disturb
f	2	d	1	no	1	during	disturb
f	2	c	1	no	1	during	disturb
f	2	d	1	no	1	during	disturb
f	2	c	2	no	1	during	disturb
f	2	d	5	no	1	during	disturb
f	2	d	5	yes	0	after	no
m	5	a	2	yes	0	before	no
m	5	b	1	no	1	before	no
m	5	a	10	no	1	before	no
m	5	c	4	no	1	before	no
m	5	d	1	no	1	before	no
m	5	e	1	yes	0	before	no
f	7	a	4	yes	0	before	no
f	7	b	1	no	1	before	no
f	7	a	1	no	1	before	no
f	7	b	1	no	1	before	no
f	7	a	1	no	1	before	no
f	7	b	11	yes	0	before	no
f	7	b	10	yes	0	during	disturb
f	7	b	2	yes	0	after	no
f	8	a	16	yes	0	before	no
f	8	b	1	yes	0	before	no
f	8	c	1	no	1	during	disturb
f	8	d	5	no	1	during	disturb
f	8	e	6	no	1	during	disturb
f	8	d	1	no	1	during	disturb
f	8	e	2	no	1	during	disturb
f	8	f	1	no	1	during	disturb
f	8	g	2	yes	0	during	disturb
f	8	g	5	yes	0	after	no
m	9	a	2	yes	0	before	no
m	9	b	1	no	1	before	no
m	9	a	1	no	1	before	no
m	9	b	1	no	1	before	no
m	9	c	1	no	1	before	no
m	9	a	1	no	1	before	no
m	9	c	1	no	1	before	no
m	9	d	1	no	1	before	no
m	9	c	2	yes	0	before	no
m	9	d	1	no	1	before	no
m	9	c	1	no	1	before	no

m	9	d	1	no	1	before	no
m	9	e	1	no	1	before	no
m	9	d	1	yes	0	before	no
m	9	f	1	yes	0	before	no
m	9	f	1	no	1	during	disturb
m	9	d	1	no	1	during	disturb
m	9	f	2	yes	0	during	disturb
m	9	f	3	no	1	during	disturb
m	9	g	1	no	1	during	disturb
m	9	h	6	no	1	during	disturb
m	9	i	2	yes	0	during	disturb
m	9	j	5	yes	0	after	no
m	10	a	1	yes	0	before	no
m	10	b	1	yes	0	before	no
m	10	a	1	yes	0	before	no
m	10	c	5	yes	0	before	no
m	11	a	5	yes	0	before	no
m	11	b	2	no	1	before	no
m	11	a	1	no	1	before	no
m	11	c	3	no	1	before	no
m	11	b	1	no	1	before	no
m	11	c	1	no	1	before	no
m	11	a	1	no	1	before	no
m	11	c	4	no	1	before	no
m	11	d	1	yes	0	before	no
m	11	c	3	no	1	during	disturb
m	11	d	1	no	1	during	disturb
m	11	c	1	no	1	during	disturb
m	11	e	1	no	1	during	disturb
m	11	d	1	no	1	during	disturb
m	11	a	1	no	1	during	disturb
m	11	e	2	no	1	during	disturb
m	11	d	1	no	1	during	disturb
m	11	e	2	no	1	during	disturb
m	11	d	1	no	1	during	disturb
f	13	a	1	yes	0	before	no
f	13	b	1	no	1	before	no
f	13	c	2	no	1	before	no
f	13	d	7	no	1	before	no
f	13	c	1	no	1	before	no
f	13	e	2	no	1	before	no
f	13	d	1	no	1	before	no
f	13	f	1	no	1	before	no
f	13	e	2	no	1	before	no
f	13	d	1	yes	0	before	no
f	13	d	1	no	1	during	disturb
f	13	e	1	no	1	during	disturb
f	13	d	1	no	1	during	disturb
f	13	g	2	no	1	during	disturb
f	13	f	8	no	1	during	disturb
f	13	h	5	yes	0	during	disturb
f	13	i	5	yes	0	after	no
m	14	a	2	yes	0	before	no
m	14	b	4	no	1	before	no
m	14	a	3	no	1	before	no
m	14	b	2	no	1	before	no
m	14	a	1	no	1	before	no
m	14	b	1	no	1	before	no
m	14	c	1	no	1	before	no
m	14	b	1	no	1	before	no
m	14	d	1	no	1	before	no
m	14	b	3	yes	0	before	no
m	14	d	1	no	1	during	disturb
m	14	e	7	no	1	during	disturb
m	14	f	1	no	1	during	disturb
m	14	e	1	no	1	during	disturb

m	14	f	1	no	1	during	disturb
m	14	g	1	no	1	during	disturb
m	14	f	1	no	1	during	disturb
m	14	g	1	no	1	during	disturb
m	14	h	1	no	1	during	disturb
m	14	g	3	yes	0	during	disturb
m	14	g	5	yes	0	after	no
f	16	a	1	yes	0	before	no
f	16	b	2	no	1	before	no
f	16	a	1	no	1	before	no
f	16	c	1	no	1	before	no
f	16	d	1	no	1	before	no
f	16	c	1	no	1	before	no
f	16	a	1	no	1	before	no
f	16	d	2	no	1	before	no
f	16	a	1	no	1	before	no
f	16	d	7	no	1	before	no
f	16	e	1	yes	0	before	no
f	16	d	1	no	1	during	disturb
f	16	e	3	no	1	during	disturb
f	16	d	9	no	1	during	disturb
f	16	f	5	yes	0	during	disturb
f	16	f	5	yes	0	after	no
f	17	a	1	yes	0	before	no
f	17	b	1	no	1	before	no
f	17	a	2	no	1	before	no
f	17	b	10	no	1	before	no
f	17	c	3	no	1	before	no
f	17	d	1	no	1	before	no
f	17	e	1	yes	0	before	no
f	17	e	10	no	1	before	disturb
f	17	f	5	no	1	before	disturb
f	17	c	3	yes	0	during	disturb
f	17	c	2	no	1	after	no
f	17	e	2	no	1	after	no
f	17		1	yes	0	after	no
m	18	a	18	yes	0	before	no
m	18	b	1	yes	0	before	no
m	18	c	4	no	1	during	disturb
m	18	d	8	no	1	during	disturb
m	18	e	6	yes	0	during	disturb
m	18	e	5	yes	0	after	no
m	19	a	1	yes	0	before	no
m	19	b	2	no	1	before	no
m	19	a	1	no	1	before	no
m	19	c	1	no	1	before	no
m	19	b	1	no	1	before	no
m	19	d	5	no	1	before	no
m	19	c	1	no	1	before	no
m	19	d	2	no	1	before	no
m	19	e	1	no	1	before	no
m	19	d	4	yes	0	before	no
m	19	d	3	no	1	during	disturb
m	19	f	3	no	1	during	disturb
m	19	g	3	no	1	during	disturb
m	19	h	6	no	1	during	disturb
m	19	g	3	yes	0	during	disturb
m	19	g	3	no	1	after	no
m	19	i	2	yes	0	after	no
f	20	a	1	yes	0	before	no
f	20	b	3	no	1	before	no
f	20	c	8	no	1	before	no
f	20	d	18	yes	0	during	disturb
f	20	d	5	yes	0	after	no
m	21	a	3	yes	0	before	no
m	21	b	1	no	1	before	no

m	21	c	1	no	1	before	no
m	21	b	8	no	1	before	no
m	21	d	1	yes	0	before	no
m	21	b	1	no	1	before	no
m	21	c	1	no	1	before	no
m	21	d	1	yes	0	before	no
m	21	b	1	no	1	during	disturb
m	21	c	1	no	1	during	disturb
m	21	b	1	no	1	during	disturb
m	21	d	2	no	1	during	disturb
m	21	b	1	no	1	during	disturb
m	21	e	1	no	1	during	disturb
m	21	f	1	no	1	during	disturb
m	21	e	3	no	1	during	disturb
m	21	b	2	no	1	during	disturb
m	21	g	1	no	1	during	disturb
m	21	e	1	no	1	during	disturb
m	21	c	1	no	1	during	disturb
m	21	g	1	no	1	during	disturb
m	21	h	1	yes	0	during	disturb
m	21	h	3	no	1	after	no
m	21	d	2	yes	0	after	no
m	22	a	1	yes	0	before	no
m	22	b	2	no	1	before	no
m	22	c	2	no	1	before	no
m	22	d	1	no	1	before	no
m	22	c	1	no	1	before	no
m	22	d	2	no	1	before	no
m	22	c	1	no	1	before	no
m	22	d	1	no	1	before	no
m	22	c	1	yes	0	before	no
m	22	d	1	no	1	during	disturb
m	22	a	4	no	1	during	disturb
m	22	e	1	no	1	during	disturb
m	22	f	3	no	1	during	disturb
m	22	g	2	no	1	during	disturb
m	22	h	1	no	1	during	disturb
m	22	i	2	no	1	during	disturb
m	22	h	1	no	1	during	disturb
m	22	j	1	no	1	during	disturb
m	22	h	1	no	1	during	disturb
m	22	g	1	yes	0	during	disturb
m	22	h	1	no	1	after	no
m	22	g	2	no	1	after	no
m	22	h	1	no	1	after	no
m	22	g	1	yes	0	after	no
m	23	a	2	yes	0	after	no
f	24	a	9	no	1	during	disturb
f	24	b	1	no	1	during	disturb
f	24	a	5	no	1	during	disturb
f	24	b	3	yes	0	during	disturb
f	24	b	5	yes	0	after	no

Supplementary Table 4.13: Table Nesting Data from Tierpark 2017

sex	individual	nest	nesttime	censor	coding
f	4	a	5	yes	0
f	4	b	26	no	1
f	4	c	5	no	1
f	4	b	1	no	1
f	4	c	1	yes	0
f	4	d	1	yes	0
m	8	a	4	yes	0
m	8	b	1	no	1
m	8	a	3	no	1
m	8	c	1	no	1
m	8	a	1	no	1
m	8	d	1	no	1
m	8	e	1	no	1
m	8	d	2	no	1
m	8	e	1	no	1
m	8	d	4	no	1
m	8	e	1	no	1
m	8	d	5	no	1
m	8	f	11	yes	0
m	9	a	1	yes	0
m	9	b	1	no	1
m	9	c	5	no	1
m	9	d	1	yes	0
m	9	c	2	no	1
m	9	a	1	no	1
m	9	c	2	no	1
m	9	a	2	no	1
m	9	e	1	no	1
m	9	f	1	yes	0
m	9	a	4	no	1
m	9	g	1	yes	0
m	9	h	1	no	1
m	9	a	1	no	1
m	9	h	12	yes	0
m	19	a	15	yes	0
m	19	b	1	no	1
m	19	a	5	no	1
m	19	b	2	no	1
m	19	a	1	no	1
m	19	b	12	yes	0
f	20	a	1	yes	0
f	20	b	1	no	1
f	20	a	1	no	1
f	20	b	1	no	1
f	20	a	1	no	1
f	20	b	9	no	1
f	20	a	1	no	1
f	20	b	23	yes	0
m	23	a	1	yes	0
m	23	b	1	no	1
m	23	a	2	no	1
m	23	b	4	no	1
m	23	c	2	no	1
m	23	a	1	no	1
m	23	c	10	yes	0
m	27	a	1	yes	0
m	27	b	9	no	1
m	27	c	1	no	1
m	27	b	2	no	1
m	27	c	17	yes	0
m	30	a	2	yes	0

m	30	b	1	no	1
m	30	a	3	no	1
m	30	c	1	no	1
m	30	a	1	yes	1
m	30	c	1	no	1
m	30	a	1	no	1
m	30	c	3	no	1
m	30	a	1	no	1
m	30	c	1	no	1
m	30	a	1	no	1
m	30	c	1	no	1
m	30	a	7	no	1
m	30	d	1	no	1
m	30	a	1	no	1
m	30	d	3	no	1
m	30	c	1	no	1
m	30	d	1	no	1
m	30	c	4	no	1
m	30	d	1	yes	0
m	31	a	1	yes	0
m	31	b	2	no	1
m	31	c	1	yes	0
m	31	d	1	yes	0
m	31	e	1	no	1
m	31	f	1	yes	0
m	31	b	1	no	1
m	31	g	2	no	1
m	31	h	2	no	1
m	31	g	1	no	1
m	31	i	1	no	1
m	31	g	1	no	1
m	31	i	1	no	1
m	31	g	5	yes	0
f	32	a	1	yes	0
f	32	b	1	no	1
f	32	c	1	no	1
f	32	b	1	no	1
f	32	d	34	yes	0
m	35	a	1	yes	0
m	35	b	1	no	1
m	35	a	1	no	1
m	35	c	1	no	1
m	35	d	2	no	1
m	35	a	1	no	1
m	35	d	2	no	1
m	35	b	1	no	1
m	35	e	2	no	1
m	35	d	1	no	1
m	35	e	12	no	1
m	35	f	1	no	1
m	35	e	2	no	1
m	35	d	3	no	1
m	35	f	3	no	1
m	35	d	1	no	1
m	35	f	2	yes	0

5 Supplementary information Chapter 5: Music festival makes hedgehogs move: How individuals cope behaviourally in response to human-induced stressors

GPS and ACC data are available through the Movebank platform (www.movebank.org) by contacting the authors. The supplementary table S3 has the data from which the SVM model was built.

Supplementary Table 5.1: Overview over Wilcoxon test results for the different parameters to compare daily values of pre-festival period with them of festival period. Significant differences are marked by bold font. KDE50 = 50% Kernel Density Estimation , DI = Diurnality Index, TSdusk = Time Span between activity onset and civil dusk, ODBA = Overall Dynamic Body Acceleration, DFC = Degree of Functional Coupling

Animal ID	sex	KDE50	DI	TSdusk	ODBA	DFC
01_2016	m	W = 223 p-value = 0.3653	W = 229 p-value = 0.03421	W = 50 p-value = 0.0007493	W = 167 p-value = 0.1486	W = 235.5 p-value = 0.2023
02_2016	f	W = 210 p-value = 0.5877	W = 212 p-value = 0.1182	W = 182 p-value = 0.5418	W = 69 p-value = 0.9771	W = 186 p-value = 0.9216
08_2016	f	W = 269 p-value = 0.008537	W = 191 p-value = 0.1093	W = 5 p-value = 3.257e-08	W 0 174 p-value = 0.001498	W = 318 p-value = 0.0003322
09_2016	m	W = 128 p-value = 0.01339	W = 108 p-value = 0.04699	W = 55 p-value = 0.3574	-	W = 128 p-value = 0.01339
13_2016	f	W = 196 p-value = 0.8786	W = 214 p-value = 0.1991	W = 49 p-value = 0.0003467	W = 98 p-value = 0.8123	W = 259 p-value = 0.02277
17_2016	f	W = 284 p-value = 0.0003577	W = 147 p-value = 0.709	W = 65 p-value = 0.05227	W = 162 p-value = 0.01007	W = 228.5 p-value = 0.2854
19_2016	m	W = 297 p-value = 0.002121	W = 132 p-value = 0.3626	W = 86 p-value = 0.02685	W = 87 p-value = 0.3134	W = 253 p-value = 0.03291
21_2016	m	W = 271 p-value = 0.02249	W = 189 p-value = 0.599	W = 82 p-value = 0.01859	W = 160 p-value = 0.1195	W = 287 p-value = 0.006603

Supplementary Table 5.2: Results for the kde 50 for all individuals. Undisturbed corresponds to the pre-festival phase, disturbed corresponds to the festival phase

Animal ID	disturbance_lvl	kde50_area	sex	ci
Igel01/2016/TP	undisturbed	3.1	m	1.5
Igel01/2016/TP	disturbed	2.14	m	0.853
Igel02/2016/TP	undisturbed	2.02	f	0.552
Igel02/2016/TP	disturbed	1.93	f	0.507
Igel08/2016/TP	undisturbed	1.99	f	0.448
Igel08/2016/TP	disturbed	1.09	f	0.456
Igel09/2016/TP	undisturbed	3.59	m	0.75
Igel09/2016/TP	disturbed	2.12	m	0.811
Igel13/2016/TP	undisturbed	1.07	f	0.292
Igel13/2016/TP	disturbed	1.04	f	0.252
Igel17/2016/TP	undisturbed	1.46	f	0.322
Igel17/2016/TP	disturbed	0.603	f	0.349
Igel19/2016/TP	undisturbed	3.02	m	0.85
Igel19/2016/TP	disturbed	1.26	m	0.513
Igel21/2016/TP	undisturbed	4.36	m	0.821
Igel21/2016/TP	disturbed	2.92	m	0.963

Raw data for the model are stored and are available from Anne Berger

Affirmation for the Doctoral Thesis/ Selbstständigkeitserklärung

I herewith declare, that I have written this thesis independently and by myself. I used no other sources, than those listed and mentioned. I have indicated where I used quotations. I assure that this thesis has not been submitted for examination elsewhere.

Ich erkläre gegenüber der Freien Universität Berlin, dass ich die vorliegende Dissertation selbstständig und ohne Benutzung anderer als der angegebenen Quellen und Hilfsmittel angefertigt habe. Die vorliegende Arbeit ist frei von Plagiaten. Alle Ausführungen, die wörtlich oder inhaltlich aus anderen Schriften entnommen sind, habe ich als solche kenntlich gemacht. Diese Arbeit wurde in gleicher oder ähnlicher Form noch bei keiner anderen Universität als Prüfungsleistung eingereicht.

Place and date Signature