The ecology of red foxes (*Vulpes vulpes*) in urban environments

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Structure of the thesis

This thesis consists of two main and two additional chapters, each of them presented as a stand-alone manuscript. The main part of the thesis focusses on the ecology of urban red foxes with both chapters dealing with their adjustments to urban environments. In chapter 1 we report on our investigation of genetic structure of foxes within Berlin and the surrounding countryside, as well as the effects of human land use on gene flow between urban and rural areas and through the urban matrix. Chapter 2 investigates habitat selection and space use patterns of red foxes radio collared within the urban area and deals with effects of the landscapes as well as the impact of human presence on movements and habitat selection. Both chapters will be discussed jointly in the general discussion of this thesis.

Chapter 3 is a methodological examination of additional assets arising from the use of the radio collars we deployed. Finally, chapter 4 presents an interdisciplinary perspective on urban foxes by investigating the perception of foxes in the general public. It uses the red fox as a model species to understand the effects of socio-demographic factors on knowledge about, risk perception of and attitudes towards wildlife. The discussion of the additional works is part of the corresponding manuscripts.

Main manuscripts

Kimmig SE., Beninde J., Brandt M., Schleimer A., Kramer-Schadt S., Hofer H., Börner K., Schulze C., Wittstatt U., Heddergott M., Halczok T., Staubach C., Frantz AC. (2019). Beyond the landscape: resistance modelling infers physical and behavioural gene flow barriers to a mobile carnivore across a metropolitan area.
Published, Molecular Ecology, 29(3), 466-484

Kimmig SE., Planillo A Wenzler-Meya M., Börner K., Brandt M., Hofer H., Kramer-Schadt S. The limits of serenity - avoidance behaviour towards humans in an opportunistic predator

Additional works

Kimmig SE/Rast W, Giese L, Berger A (2020). Machine learning goes wild: Using data from captive individuals to infer wildlife behaviours.
Published, PloS one, 15(5), e0227317

Kimmig SE, Flemming D, Kimmerle J, Cress U, Brandt M (2020): Sociodemographic aspects of wildlife-tolerance: Knowledge, risk perception and attitude towards red foxes (*Vulpes vulpes)* in Germany.
Published, Conservation Science and Practice, 2(7), e212

Content

Zusammenfassung

Die globalen Landveränderungen durch den Menschen stellt eine immense Bedrohung für natürliche Ökosysteme dar, mit vielfältigen Folgen für Wildtier-Populationen. Landveränderungen dienen unter anderem der landwirtschaftlichen Versorgung der menschlichen Bevölkerung, dem Ressourcengewinn und der industriellen Produktion, sowie der Schaffung von Wohnraum. Die weltweite Ausbreitung städtischer Räume schafft veränderte, menschlich überformte und somit neuartige Ökosysteme für Wildtierarten. Letztere überlebten entweder in von der Urbanisierung eingeschlossenen Resten ursprünglicher Habitate oder besiedelten Stadtgebiete aktiv. Diese neuartigen Lebensräume bieten hohe Nahrungsdichten und vielfältige Brutmöglichkeiten, sind jedoch durch ein hohes Maß an menschlicher Störung, Lärm- und Lichtverschmutzung sowie Habitat-Fragmentierung gekennzeichnet. Die Fähigkeit, mit diesen Umgebungsbedingungen zurechtzukommen, hängt von der phänotypischen Plastizität oder ökologischen Flexibilität von Arten (und Individuen) innerhalb der bestehenden Merkmalsausstattung ab, oder alternativ von einer sehr schnellen Evolution, die neue genetische Grundlagen für neue Merkmale als evolutionäre Anpassungen an das Stadtleben liefert. Dem Rotfuchs (*Vulpes vulpes*) als Allesfresser mittlerer Größe mit einer breiten geografischen Verteilung ist es gelungen, Städte auf der ganzen Welt erfolgreich zu besiedeln. Aufgrund ihrer allgegenwärtigen Präsenz in vom Menschen dominierten Landschaften, von landwirtschaftlichen Flächen bis zu dicht bebautem Gebiet, wird gemeinhin davon ausgegangen, dass Rotfüchse gut mit menschlicher Präsenz zurechtkommen. Obwohl es die Flexibilität der Art offensichtlich ermöglichte, diese Lebensräume zu besiedeln, geht das Leben in der Nähe des Menschen auch mit Herausforderungen für den Fuchs einher. Hohe Sterblichkeitsraten, niedrige durchschnittliche Lebensdauern und heimliche Verhaltensweisen deuten auf Einschränkungen für diesen ursprünglich scheuen Generalisten hin, die unzureichend untersucht sind. Treffen wir also anhand oberflächlicher Feststellungen falsche Aussagen über das wahre Ausmaß der phänotypischen Plastizität der Füchse?

Um diese Frage zu beleuchten, analysierten wir (i) mittels genetischer Proben entlang eines rural-urbanen Gradienten die genetische Struktur der Füchse auf Populationsebene. Wir untersuchten, inwiefern das urbane Umfeld den Genfluss innerhalb und zwischen den Fuchspopulationen in Stadt und Land beeinflusste und wie die städtische Umgebung diesen jenseits von einzelnen Landschaftselementen möglicherweise beeinträchtigt hat. (ii) Des Weiteren erforschten wir die individuelle Lebensraumnutzung von Füchsen mit Hilfe der Besenderung von Tieren im Berliner Stadtgebiet. Wir untersuchten insbesondere, wie Füchse auf verschiedene Landschaftstypen - einschließlich menschlicher Strukturen wie bebauter Flächen und Verkehrsadern - reagierten und mit unterschiedlichen Ausmaßen an menschlicher Präsenz und Aktivität umgingen.

Die Ergebnisse des ersten Kapitels zeigen, dass der Genfluss der Füchse zwischen Berlin und Brandenburg begrenzt war und zwei Populationen bestanden, die genetische differenzierbar waren. Obwohl Landschaftselemente dabei einen gewissen Einfluss auf den Genfluss ausübten, schienen sie für die Abwanderungsrouten der Füchse eine eher untergeordnete Rolle zu spielen. So hatten dicht bebaute Gebiete nur schwache negative Auswirkungen auf den Genfluss und auch Grünflächen wie Stadtparks und Wälder trugen nur wenig zum Genfluss bei. Füchse vermieden es, die Stadtgrenze zu überqueren und migrierten trotz des inhärenten Mortalitätsrisikos überwiegend entlang von Transportinfrastruktur wie Autobahnen und Eisenbahnlinien. Dies weist darauf hin, dass Vermeidungsverhalten gegenüber dem Menschen das Migrationsverhalten der Füchse mitbestimmt.

Im zweiten Kapitel berichten wir über die Raumnutzung der Füchse und verglichen dafür den aktiv genutzten mit dem insgesamt verfügbaren Lebensraum. Die Ergebnisse zeigten, dass Füchse dicht bebaute Gebiete oder Gebiete mit einem hohen Versiegelungsgrad nicht mieden, obgleich hohe menschliche Bevölkerungsdichten durchaus vermieden wurden. Die Füchse nutzten ferner nicht vermehrt Grünflächen wie öffentliche Parks oder städtische Wälder. Bevorzugt genutzt wurden Brachflächen - inklusive der Flächen entlang von Bahntrassen - und Gärten von Einfamilienhäusern und Wohnhäusern, die für die Öffentlichkeit entweder unzugänglich oder mit geringer menschlicher Präsenz assoziiert sind. Schließlich war die Meidung hoher menschlicher Bevölkerungsdichten innerhalb typischer menschlicher Aktivitätszeiten ausgeprägter. Die Ergebnisse legen nahe, dass die Raumnutzung der Füchse durch Vermeidungsverhalten gegenüber dem Menschen (mit)bestimmt wurde.

Obwohl Füchse in städtischen Lebensräumen anscheinend gut zurechtkommen, konnten wir zeigen, dass menschliche Präsenz Konsequenzen für die Tiere auf individueller und auf Populationsebene hat. Lokale und zeitliche Aktivitäten der Menschen drängen Füchse zur Anpassung ihrer Bewegungsmuster bei der Nutzung des städtischen Lebensraums. Die Ergebnisse zeigen somit die Grenzen der phänotypischen Plastizität dieser Art auf. Wir hoffen, dass unsere Erkenntnisse dazu führen werden, diverse Faktoren jenseits von Landschaftstypen in Studien zur Ökologie der Wildtiere besser zu berücksichtigen.

Weitere Arbeiten

Für die Untersuchung des Raumnutzungsverhaltens verwendeten wir VHF-Sender, die einen tri-axialen Beschleunigungssensor enthalten, der Bewegungen der Messeinheit im dreidimensionalen Raum misst. Da diese das theoretische Potenzial zur Fern-Detektion von Verhaltensweisen liefern, führten wir dazu eine methodische Studie durch (drittes Kapitel). Wir besenderten Füchse in Gefangenschaft und dokumentierten ihr Verhalten während der Messungen, um damit anschließend verschiedene Maschine Learning Programme darin zu trainieren, Verhaltensweisen aus den Beschleunigungsdaten abzuleiten. Dabei konnten wir zeigen, dass neuronale Netzwerke, besser als herkömmliche Ansätze, das Potential besitzen mithilfe von Beschleunigungsdaten Verhaltensweisen der Tiere zu klassifizieren.

Die Anwesenheit von Füchsen in städtischen Gebieten ist auch für die Bevölkerung von Interesse. Neben der möglichen Übertragung von Krankheiten verursachen Füchse Sachschäden an privatem und öffentlichem Eigentum und verursachen Störungen (z. B. durch Gerüche oder Geräusche). Kurze Fluchtdistanzen und ungewohnte Annäherung der Tiere an Menschen (oft in Gärten und manchmal gar in Häusern) schüren zudem Ängste in der Bevölkerung, führen zu Ärger und können in der Forderung nach einer Populationskontrolle städtischer Fuchspopulationen gipfeln. Um besser zu verstehen, welche Faktoren die Wahrnehmung von Wildtieren beeinflussen, führten wir eine repräsentative Umfrage durch (viertes Kapitel). Wir fanden heraus, dass Einstellung und Risikowahrnehmung gegenüber Füchsen maßgeblich die Haltung der Teilnehmenden beeinflusste, ob und welcher Form populationsbeeinflussenden Maßnahmen durchgeführt werden sollten, während Fachwissen dies nicht tat. Einstellung und Risikowahrnehmung hingen dabei hauptsächlich vom Bildungsgrad, Alter, Geschlecht und der Wohnumgebung der Teilnehmenden ab.

Summary

Global human land alteration poses an immense threat to natural ecosystems with consequences to wildlife populations on numerous scales. Land is altered for agricultural supply of the human population, the gain of resources, and industrial production as well as for providing living space. The sprawl of urban agglomerations around the world creates novel ecosystems for wildlife species that remained in natural remnants enclosed by urbanization processes or actively colonized urban areas. These novel habitats provide high food abundances and diverse breeding opportunities but are also characterized by a high degree of human-induced disturbance, land- and light pollution and habitat fragmentation. The capacity to adjust to these novel environmental conditions depends on the behavioural plasticity or ecological flexibility of species (and individuals), or on rapid evolutionary processes that provide the genetic base for adaptive trait changes. The red fox (*Vulpes vulpes*) as a generalist predator of medium size and a broad geographic distribution, managed to successfully inhabit cities around the globe. Due to their ubiquitous presence in human dominated landscapes, ranging from agricultural land to densely built-up areas, it is commonly assumed that red foxes cope well with human presence. Although the fox's inherent behavioural plasticity obviously enables the species to populate those areas, living in close proximity to humans may come with some downsides too. High mortality rates, low average life spans and elusive behaviours indicate a trade-off for this naturally shy generalist that is poorly addressed. Do we thus draw wrong conclusions about the actual boundaries of the behavioural plasticity of red foxes, based on shallow observations?

To address this issue we (i) looked at genetic patterns on the population level by analysing red fox samples across a rural to urban continuum. We investigated how the urban matrix affected gene flow in foxes and how the urban environment potentially shaped the red fox population genetics beyond the effects of single landscape elements. We then (ii) researched space use of foxes on an individual level by radio-collaring individuals across the Berlin area. We examined how foxes adjust their habitat use within the city depending on landscape - including manmade structures such as built-up areas and traffic infrastructure - as well as on human presence and activity.

The results of the first chapter revealed that gene flow between urban and rural fox population of Berlin and Brandenburg was limited, resulting in two genetically separable populations. Landscape did effect gene flow through the urban matrix to a certain extent but seemed to play a minor role for fox dispersal.

For instance, while built-up areas had only weak impeding effects on gene flow despite their high degree of urbanization, urban green spaces like city parks and forests did not serve as gene flow enhancement either. Foxes avoided crossing the city border and predominantly dispersed along urban transport infrastructure such as larger streets and railways, despite the inherent mortality risk. This indicates that also human-induced fear drives dispersal behaviour in the studied red fox population.

The second chapter reports on movement and space use of the foxes based on the comparison of used to available habitat. The results show that foxes did not avoid built-up areas or high degrees of imperviousness (ground sealing), while high human population densities were avoided. The foxes further did not preferentially select green spaces like public parks or urban forests. Wasteland areas - including verges along railways - and gardens of residential houses were predominantly used by the studied individuals, providing sites inaccessible to humans or with low human presence. Finally avoidance of humans was more distinct during times of human activity. The results pinpoint that the foxes' space use was partly driven by avoidance behaviour towards humans.

Our study showed that although foxes cope well with the urban landscape as a species, human presence has consequences on a population level and on an individual scale. Human local and temporal activities pushed the foxes into an adjustment of movement patters and their use of the urban habitat. The results also revealed the limits of this adjustment even in a flexible species like the red fox. We hope that our findings enhance the consideration of multiple factors beyond landscape for future studies on the ecology of wildlife.

Additional works

For studying space use behaviour, we used radio collars that include a tri-axial accelerometer that measures deflections of the unit within the three-dimensional space. As recorded acceleration data hold an understudied potential to analyse animal behaviour using remote tracking, we also included a methodological work into our project (third chapter). We radio collared captive foxes and documented the behaviours they displayed during measurement, to train different machine learning programs in the inference of behaviours from the acceleration data. We showed that neural networks may provide an improved ability for the classification of animal behaviours from acceleration data using machine learning compared to established approaches.

The presence of foxes in urban areas also concerns people. In addition to the possible transmission of diseases, foxes cause damage on private property and in public spaces and induce disturbances (e.g. due to odours or noises). Short fleeing distances and unfamiliar approaches of the animals (in gardens and sometimes even houses) stir up fears in the population, but can also create annoyance and calls for control of the urban fox population. We therefore conducted a representative survey to look more closely into the factors affecting wildlife perception (fourth chapter). We found that attitude towards and risk perception of foxes mainly influenced the participants' preferences on whether and how to deal with the fox population, while factual knowledge did not influence their positions. Risk perception and attitude mainly depended on education, age, gender and living environment of the participants.

General introduction

The human footprint and its implications for wildlife

The human footprint on earth is large and ubiquitous. The entire global ecosystem is in a critical environmental transition on a planetary scale that is threatening the sustainability of life on earth, including humans (Barnosky et al., 2012; Vitousek et al. 1997). The latter concerns, amongst others, the scarcity of critical resources, the degradation of ecosystem services, and the erosion of the planet's capability to cope with human waste (Steffen et al., 2011). Worldwide changes to former natural forests, green spaces and waterways undermine the capacity of ecosystems to sustain food production, maintain healthy freshwater bodies and forests, regulate climate and air quality, and ameliorate infectious diseases (Foley et al., 2005). Humans have largely altered the face of the earth (Fig. 1) and global land use - including croplands, pastures, plantations, and urban areas, accompanied by large increases in energy, water and fertilizer use - results in a critical loss of biodiversity (e.g. Brook et al., 2003). Up to half of the planet's species are predicted to be lost within in the next 50 years (Thomas et al., 2004). For example, approximately one quarter of all living (marine and terrestrial) mammals worldwide is at risk of extinction to date (Schipper et al., 2008) and more than 75% of insect biomass has been lost within less than 30 years in protected areas in Germany (Hallmann et al., 2017).

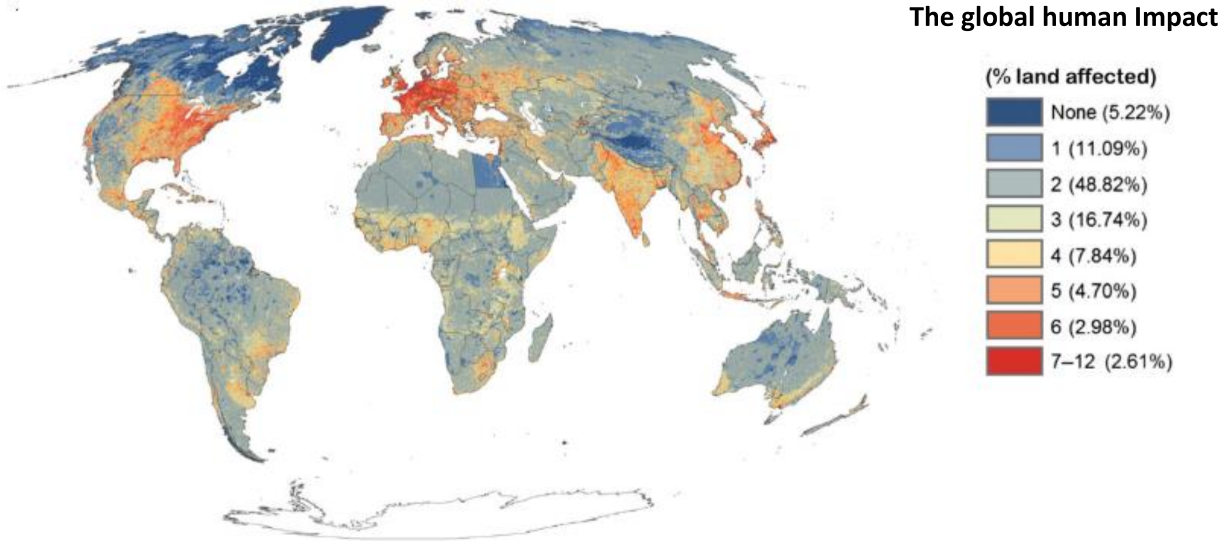

Figure 1: Spatial distribution of thirteen overlapping human induced stressors per 1-km² area, and the percentage of terrestrial lands affected globally (in parentheses). Modified from Kennedy et al. 2019.

One crucial aspect of human land alteration is urbanisation: During the past 100 years, a rapid transition of natural land into urban agglomerations as well as the expansion of existing urban areas has led to the majority of the world's population living in cities (Heisler & Brazel, 2010) and the urbanisation process is predicted to increase further (Ritchie & Roser, 2018), with global consequences for wildlife. The encroachment of cities into natural habitats around the world represents a permanent loss of natural habitat needed for wildlife species (Miller & Hobbs, 2002) but it also leads to an "urbanisation" of various wild animal species. Compared to sprawling agricultural monocultures, urban areas provide heterogeneous structures and harbour a variety of ecological niches for animals. Therefore it is often argued that urban areas may be essential to actually conserve biodiversity, given the inadequacy of natural protected area systems (Lundholm & Richardson, 2010). However, living in urban areas effectively means that animals mostly dwell in 'novel habitats' - habitats characterized by ecological conditions that may substantially differ from conditions in natural habitats (Lundholm & Richardson, 2010; Pickett et al., 2001). Urban related ecological conditions that may affect life history traits of animals, for example, concern increased temperatures within the cityscape (Heisler & Brazel, 2010), intense habitat fragmentation (Fahrig, 2003; Gibb & Hochuli, 2002), noise pollution, light pollution by artificial night lighting (Katz & Levin, 2016; La Sorte et al., 2017) and also the influence of human activities (Rabele, 1994; Shochat et al., 2006a) as well as the density and activities of their companion animals (Lenth et al., 2008; Plaza et al., 2019). Consequently, the urban habitat represents a major challenge for the adaptive capacities of a wild animal species (Ditchkoff et al., 2006). As habitat changes due to human land alteration and urbanization occurred on a short ecological rather than a longer-term evolutionary time scale, it is likely that urbanisation tests the limits of the tolerance (phenotypic plasticity) or adaptability of species to cope with such conditions (Smith et al., 2018). This makes urban areas an ideal setting to study phenotypic plasticity and resilience in the behaviour of wildlife.

The presence of wild animals in urban areas also gives rise to potential conflicts with their human inhabitants (Distefano, 2005). Amongst others, the implications of the presence of urban wildlife for people include the transmission of zoonoses (Ahmed et al., 2019; Morse et al., 2012), perceived risks, including fear regarding direct encounters (Carter et al., 2020; Hanisch-Kirkbride, at al., 2013), loss of livestock, companion animals and damages to property (Bagchi & Mishra, 2006; Czech et al., 2000) but also "recreational" aspects, e.g. positive aspects for people through the delights of encountering wild species (Cordingley et al., 2016; Rockel & Kealy, 1991). It would therefore be of great interest to establish a framework to predict the potential effects of urbanisation measures on wildlife populations, not only with regard to conservation of biodiversity but also for the purpose of resolving human-wildlife conflicts (Baker & Harris, 2007; McKinney, 2006). The ecological discipline of urban ecology which started as recently as the 1970s (McDonnell, 2011) has become an established discipline in ecological research accordingly, with a steadily increasing number of scientific publications (McDonnell, 2015; Weiland and Richter, 2009). It pursues a practical impact by making cities more attractive, more enjoyable, and better for the health of people and that of the planet (Goode et al., 2020) but also pursues understanding the fundamental principles of adjustment and adaptation of species to changing ecosystem conditions.

Despite the growth of this discipline and although an increasing number of species is found in urban habitats, for many species the level of knowledge is still poor about the degree of tolerance to urban conditions (known as phenotypic plasticity) and the adjustment mechanisms of wild animals (Magle et al., 2012). Species substantially vary regarding their behavioural response to human proximity, many species cannot persist in human dominated areas, whereas others cope well and even flourish in the novel ecosystems (Møller, 2009; Shochat et al., 2006b). The extent to which urban dwelling animals apparently adjust their ecology and behaviour to urban areas is often used to categorise such wildlife (Fischer et al., 2015), e.g., in “urban avoiders”, “urban utilisers” and “urban dwellers”. Urban avoiders like the mountain lion (*Puma concolor,* Gehrt et al., 2010) are species that reach their highest densities in the most natural sites (Blair 1996) such as remnants of natural habitats (Markovchick-Nicholls et al., 2008) and that rarely occur in developed areas (Fischer et al. 2015). Fischer et al. (2015) based their definition of urban dwellers and urban utilisers on the relative importance of natural and developed areas to their population dynamics. Following their classification system, urban dwellers are species whose persistence in an urbanised landscape is independent of natural areas, e.g., the orb-weaving spider (*Nephila plumipes*, Lowe et al. 2014). Urban utilisers only occur in urban environments as non-breeders or as breeders that are present only because of dispersal from adjacent natural areas as in the case of the northern brown bandicoot (*Isoodon macrourus,* FitzGibbon et al., 2007). The exact definition of such classifications as well as its terminology varies between authors and the use of “urban adapters” or “urban exploiters” as categories is also common (see Blair, 1996; McKinney, 2002). The boundaries of these classifications are often fluid and some species might be difficult to assign to one specific category. What these attempts at such classifications certainly demonstrate is that wildlife clearly shows different capacities to adjust to the urban habitat.

Red fox plasticity

Mammalian urban dwelling species in Europe include bats, hedgehogs, mice, voles, the racoon (*Procyon lotor)*, the Eurasian badger (*Meles meles*), the European otter (*Lutra lutra*) and others (Baker & Harris, 2007; Bateman & Fleming, 2012). One species that successfully inhabits cities and urbanised areas is the red fox (*Vulpes vulpes*). The phenomenon of the city fox was first observed in Great Britain in the 1970s (Harris & Rayner, 1987; Soulsbury et al., 2011; White et al., 1996) and it was scientifically documented for various British cities such as London, Bristol or Oxford (e.g., Baker et al., 2000; Harris, 1979, 1981; Iossa et al., 2008; Trewhella et al., 1988). As red foxes inhabit urban areas in numerous countries, urban fox populations have been studied in many other places around the world, for example in Belarus (Sidorovich et al., 2006), in Sapporo, Japan (Uraguchi et al., 2009), or in Zurich and Geneva in Switzerland (Contesse et al., 2003; Gloor, 2002; Fischer et al., 2005). For several decades, the red fox is also established in major German cities.

For mesocarnivores like the red fox, urban areas provide a variety of potential spatial niches, including streets, parks and other public green spaces, squares and built-up areas, all with a different degree of artificiality and human disturbance (Bateman & Fleming, 2012). These areas may be used by the animals for different purposes, for instance green areas may provide shelter and suitable den sites (Adkins & Stott, 1998; Baker et al., 2000; Baker & Harris, 2007), railways, streets and other linear features may serve as corridors for movements (Lewis et al., 1993). In addition to urban areas, red foxes also inhabit a large variety of different natural habitat types. Their natural range covers the hot deserts of North Africa, the Middle East and the Mediterranean as well as the temperate climate zone and the snowy landscapes in the global north, including Siberia, Scandinavia and North America. The species' geographical distribution makes the red fox the most wide-spread terrestrial wild carnivore on the planet (Schipper et al., 2008). It therefore displays an impressive level of apparent adaptability and flexibility with regard to its habitat requirements such as choice of sleeping or breeding space, diet and even social structure (Baker et al., 2004; Bateman & Fleming; 2012, Iossa et al., 2009).

The special ability of the red fox to populate diverse environments is therefore a key element in research into how wild animals adjust to new habitats, including novel urban habitats. Furthermore, because it is abundant in many places, the red fox may serves as a model species to provide insights into the adjustment strategies of animals that may benefit the protection of rare and threatened wild animal species, for which small population sizes and their threat status may limit research opportunities. The abundance of red foxes also harbours a certain potential for conflict. For example, regarding its role as a potential carrier of zoonoses such as rabies and as host of the small fox tapeworm (*Echinococcus multilocularis*) as well as other infectious diseases of relevance to companion or domestic animals such as canine distemper virus (CDV) or sarcoptic mange. While rabies is officially extinct in Germany and most parts of central Europe, infections with alveolar echinococcosis caused by the fox tapeworm are dangerous to humans although rare (Combes et al., 2012). This parasite was for example detected in Geneva and Zurich, Switzerland with varying levels of prevalence in city foxes (Fischer et al., 2005; Hofer et al., 1999). Finally, the potential to study evolutionary processes in a species with high phenotypic plasticity may be of interest to scientists asking questions about fundamental evolutionary process, with urbanisation being considered a quasi-experimental setup of an extreme environment. Consequently the species has been in the focus of early urban ecological research, with Stephen Harris and his colleagues in Bristol and David Macdonald and his colleagues in Oxford studying the ecology and behavior of red foxes in settlement areas in the 1970s.

The results of these and subsequent studies show that red foxes reach high densities in urban areas with tenfold to fifteenfold higher population densities than in rural areas (e.g. Chautan et al., 2000; Harris, 1981; Trewhella et al., 1988). The urban environment appears to have certain advantages for an opportunistic omnivore. For instance, red foxes and other mesocarnivores may benefit from human activities by the ability to exploit anthropogenic resources (Newsome, 2015) due to a certain inherent tolerance of these species towards human presence (McKinney, 2006). Besides high food availability and density (Contesse et al., 2003), the absence of natural apex predators could play a role here (Baker & Harris, 2007; Bateman & Fleming, 2012; Crooks & Soulé, 1999). On the down side, living in the city may also involve fitness costs. For example, fox mortality within cities is particularly high from road traffic (Baker et al., 2007) and in case of disease outbreaks, high urban fox population densities accelerate rates of intraspecific transmission (Baker et al., 2000). Especially sarcoptic mange and canine distemper virus (CDV) outbreaks can kill large proportions of a red fox population within a short time. For instance, Soulsbury at al. (2007) reported a short-term, mange-induced decline in fox density in Sweden of up to 95%. Human presence may also negatively influence red fox behaviour - mesocarnivores were observed to display a diverse range of behavioural responses to human presence and human activity (Barrueto et al., 2014; Sévêque et al., 2020; Wang et al., 2015; Wilmers et al., 2013).

Objectives & purpose of this study

This study focused on human-induced limitations to the ability of red foxes to operate within an urban setting, by considering two key aspects of life history traits: (1) their population structure in terms of genetic exchange and dispersal opportunities, (2) their use of and movement through space.

(1) Urban areas are known to restrict animal movements and thus limit the functional connectivity - the connectivity of the landscape from the species perspective (Tischendorf & Fahrig, 2000) - of the urban environment. The urban matrix is thus likely to have an impact on a species' ability to disperse (Bohonak, 1999). It has been shown for numerous species that urban populations are affected by geographical barriers and may become reproductively isolated (e.g. Gortat et al., 2017; Lourenço et al., 2017). Physical barriers often predominantly concern small and moderately mobile species (Beninde et al., 2018; Combs et al., 2018; Munshi-South, 2012). Apart from physical barriers, however, human presence and activities may also restrict animals in urban areas if the species avoids humans which may often be perceived as a threat (Samia et al., 2015). If sufficiently common, such behaviour-related restrictions in movement capacity should impede genetic exchange amongst individuals (possibly resulting in distinct genetic subpopulations), even in a mobile species such as the red fox which is physically capable of crossing manmade barriers such as streets, railway lines or freshwater bodies such as rivers (Adkins & Stott, 1998).

To address the effect of the urban matrix on red fox populations we studied the population genetic structure as well as gene flow patterns across a rural to urban gradient. We asked whether urban and rural populations are genetically distinct, and how different landscape elements may affect red fox dispersal patterns, testing three different hypotheses:
(1.1) Due to their inherent plasticity and mobility, red foxes disperse unhampered throughout the city. This predicts that urban and adjoining rural populations are panmictic, the urban fabric has no influence on gene flow and there should be no population or landscape genetic structure.

(1.2) Red fox dispersal is solely affected by physical barriers such as rivers, built-up areas and highways. This predicts that multiple physical barriers limit gene flow, resulting in several scattered genetic populations with a distribution concomitant to major physical barriers, as revealed by a landscape genetic analysis.

(1.3) Red fox dispersal is primarily affected by behavioural barriers. This predicts that there is population structure and that dispersal is limited by the city border where the rural landscape transits to the urban environment. Accordingly, two major genetic subpopulations are predicted and landscape related factors beyond physical barrier effects (such as disturbance levels) are expected to affect gene flow within city boundaries.

(2) An animal's use of space and habitat selection emerges from its movement patterns, which are, in turn, determined by their behavioural or physiological states and by extrinsic factors (Karelus et al., 2019). Human presence may be such an extrinsic factor, affecting urban wildlife movement. In their review of movement studies worldwide, Tucker et al. (2018) concluded that movements of mammals in areas with a comparatively high human footprint were on average one-half to one-third the extent of their movements in areas with a low human footprint. Such decreasing animal movements may arise from habitat fragmentation, barrier effects, or an increase in resource availability (Prange et al., 2004; Said et al., 2005; Sawyer et al., 2013). Whereas movement distances may become shorter in environments with higher resources, some studies also report longer movements as a result of habitat loss, habitat fragmentation or altered movement routes (Lenz et al., 2011; Tigas et al., 2002). Either way, human activity may limit animal movements (and space use accordingly), and therefore influence the behaviour and ecology of urban red foxes, for example by restricting their foraging opportunities or increasing their mortality.

We therefore studied space use patterns in response to different landscape features as well as to human population density (including human activity times) within the urban matrix. We asked which of these factors influence red fox habitat selection, testing the three following hypotheses:

(2.1) Red fox behavioural plasticity and adjustment to urban environments enables it to fully exploit the urban area. This predicts that foxes should use the urban landscape evenly, with little evidence for avoidance or preference for specific landscape elements, and human population density should not affect red fox movement.

(2.2) Red foxes rely on green vegetation areas i.e., natural-like habitats. This predicts that they avoid built-up areas and grey spaces (like streets and places) and specifically select green spaces, including public green spaces and forested areas. Again, human population density should have no effect.

(2.3) Red foxes adjust their use of space to human presence and activity times. This predicts that foxes show preference or avoidance behaviour to covariates associated with human presence such as an avoidance of densely populated areas, a preference for sites that are inaccessible to humans (such as wasteland), and a preference for undisturbed habitat at times of overall increased human activity.

Our results may enhance the understanding of the opportunities as well as the limits of adjustment to urban environments in a human commensal species and elucidate the underlying mechanisms of emerging patterns in red fox behaviour.

MAIN CHAPTERS

Chapter 1: Population and landscape genetics along an urbanisation gradient

Beyond the landscape: resistance modelling infers physical and behavioural gene flow barriers to a mobile carnivore across a metropolitan area

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Author contribution

The original idea to compare urban and rural fox populations was developed by Heribert Hofer (HH), Miriam Brandt (MB) and Stephanie Kramer-Schadt (SKS). The detailed concept and the embedding of the genetic analysis in a broader ecological context was elaborated by Sophia E. Kimmig (SEK). The methodological approach and the extension of the study by landscape genetic analysis was decided by Alain C. Frantz (ACF) and SEK. The collection of samples was done in a cooperation with Konstantin Börner (KB) and Mike Heddergott (MH) and a cooperation of SEK with Ulrich Wittstatt (UW). Anna Schleimer (AS) and Tanja Halczok (TH) conducted laboratory work. ACF and SEK selected landscape features, Christoph Staubach (CSt) and Joscha Beninde (JB) contributed to the generation of the resistance surfaces. ACF conducted landscape genetic analysis under the assistance of SEK. SEK conducted population genetic analysis. SEK wrote the manuscript. ACF, HH, JB, MB, SKS contributed with substantial revisions to the manuscript.

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Beyond the landscape: Resistance modelling infers physical and behavioural gene flow barriers to a mobile carnivore across a metropolitan area

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Abstract

Urbanization affects key aspects of wildlife ecology. Dispersal in urban wildlife species may be impacted by geographical barriers but also by a species' inherent behavioural variability. There are no functional connectivity analyses using continuous individual-based sampling across an urban-rural continuum that would allow a thorough assessment of the relative importance of physical and behavioural dispersal barriers. We used 16 microsatellite loci to genotype 374 red foxes (*Vulpes vulpes*) from the city of Berlin and surrounding rural regions in Brandenburg in order to study genetic structure and dispersal behaviour of a mobile carnivore across the urban-rural landscape. We assessed functional connectivity by applying an individual-based landscape genetic optimization procedure. Three commonly used genetic distance measures yielded different model selection results, with only the results of an eigenvector-based multivariate analysis reasonably explaining genetic differentiation patterns. Genetic clustering methods and landscape resistance modelling supported the presence of an urban population with reduced dispersal across the city border. Artificial structures (railways, motorways) served as main dispersal corridors within the cityscape, yet urban foxes avoided densely built-up areas. We show that despite their ubiquitous presence in urban areas, their mobility and behavioural plasticity, foxes were affected in their dispersal by anthropogenic presence. Distinguishing between man-made structures and sites of human activity, rather than between natural and artificial structures, is thus essential for better understanding urban fox dispersal. This differentiation may also help to understand dispersal of other urban wildlife and to predict how behaviour can shape population genetic structure beyond physical barriers.

KEYWORDS

dispersal, functional connectivity, landscape of fear, landscape resistance modelling, RESISTANCEGA, urban ecology

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1 | INTRODUCTION

Urbanization results in dramatic environmental change (Johnson & Munshi-South, 2017) and some species flourish in these semi-artificial ecosystems (Møller, 2009; Shochat, Warren, Faeth, McIntyre, & Hope, 2006). Yet, urbanization affects key aspects of wildlife ecology such as survival, foraging and reproductive success (Wilson et al., 2016). Moreover, it has a substantial impact on the movement ecology and dispersal ability of populations (Johnson & Munshi-South, 2017; Tucker et al., 2018). Urban species may for example be restricted by geographical barriers and become reproductively isolated (Gortat, Rutkowski, Gryczynska, Kozakiewicz, & Kozakiewicz, 2017; Lourenço, Álvarez, Wang, & Velo-Antón, 2017). The physical structure of the urban environment is thus likely to have an impact on dispersal capacity (Bohonak, 1999).

However, the urban environment imposes much more on wildlife than the need to navigate a highly altered landscape. Animals often perceive humans as predators and avoid areas of human activity (Samia, Nakagawa, Nomura, Rangel, & Blumstein, 2015). Individuals from the rural surroundings of an urban area might thus face a behavioural barrier to enter urbanized areas. Within the city, species with the physical capability of crossing the urban matrix may face behavioural barriers if they avoid man-made objects (with their artificial structures or scents) or human presence per se. Different scenarios are thus conceivable for population structure and drivers of gene flow across the urban-rural continuum and the perception of human-induced risks may differentiate urban and rural populations beyond physical barriers. The role of behavioural limitations to movement has been frequently overlooked. Examining the functional connectivity—the connectivity of the landscape from the species' perspective (Tischendorf & Fahrig, 2000)—of the urban landscape would thus help to assess the relative importance of physical and behavioural dispersal barriers and thereby make an important contribution to understanding the ecology and evolution of wildlife in novel environments.

Molecular genetic methods permit inferences about wildlife dispersal without the need to collect extensive field data on individual movements (Frantz, Do Linh San, Pope, & Burke, 2010; Guillot, Leblois, Coulon, & Frantz, 2009). Recently, numerous studies of gene flow in urban areas have been published, but many of those focus on smaller and less mobile species that are thought to face major barriers in urban areas (Beninde, Feldmeier, Veith, & Hochkirch, 2018; Combs, Puckett, Richardson, Mims, & Munshi-South, 2018; Munshi-South, 2012). Studies on larger and more vagile species, in contrast, analysed the population genetic structure of animals from peripheral suburban populations or from isolated sampling sites within urban and rural areas (Blanchong, Sorin, & Scribner, 2013; Santonastaso, Dubach, Hauver, Graser, & Gehrt, 2012; Stillfried, Fickel, et al., 2017; Wandeler, Funk, Largiadèr, Gloor, & Breitenmoser, 2003). There is currently no thorough analysis of the population and landscape genetic structure of a vagile species in an urban-rural continuum available, using continuous individual-based sampling. This would permit to identify drivers of urban gene flow, including those unrelated to the physical properties of the landscape.

Landscape genetic methods are particularly suited to assess functional connectivity. Specifically, hypotheses on how the environment influences gene flow in a target species can be evaluated by statistically relating the distribution of genetic similarities among individuals to landscape characteristics (Cushman, McKelvey, Hayden, & Schwartz, 2006; Schwartz et al., 2009). Several statistical problems have been recently solved, such as the nonindependence among ecological distances and the subjective assignment of resistance values to environmental features (Peterman, 2018; Prunier, Colyn, Legendre, Nimon, & Flamand, 2015; Sawyer, Epps, & Brashares, 2011). Landscape genetic approaches are still evolving (Balkenhol, Waits, & Dezzani, 2009; Manel & Holderegger, 2013; Richardson, Brady, Wang, & Spear, 2016) and some methodological aspects remain relatively underexplored. For example, while a simulation study by Shirk, Landguth, and Cushman (2017) has suggested that not all genetic distance measures perform equally well in model selection, different genetic distance measures have not been tested with the same empirical data set.

Aiming to gain a more fundamental understanding of the impact of urbanization on wildlife populations at a large spatial scale, we here focus on a mobile mesopredator, the red fox (*Vulpes vulpes*). Red foxes are ecologically flexible (Voigt & Macdonald, 1984) and occur in various habitat types. Their populations prosper even in highly urbanized habitats. In Berlin, our focal city, the first reports of foxes date from the 1950s (Saar, 1957) and by the 1990s the entire city was populated (Börner, Wittstatt, Schneider, 2009). Their ubiquitous distribution in highly artificial and fragmented areas as well as their movement ecology make foxes an ideal model for this study. On the one hand, foxes are very mobile. Urban animals have been reported to routinely cross streets and even rivers (Adkins & Stott, 1998) and gene flow may be unhampered by the urban landscape. On the other hand, anthropogenic infrastructure could represent significant gene flow barriers for mobile carnivores (Riley et al., 2006) and both telemetry and genetic studies point towards the existence of distinct urban and rural fox populations (Janko et al., 2011; Wandeler et al., 2003).

Here, we used continuous sampling of individuals both within Berlin as well as the adjoining rural countryside to evaluate three hypotheses. (a) The null hypothesis was that, due to their niche breadth and mobility, foxes disperse unhampered throughout the city and urban and adjoining rural populations are panmictic. This predicts that the urban fabric has no influence on gene flow, resulting in the absence of population and landscape genetic structure. (b) The fragmentation hypothesis posits that fox dispersal was (solely) affected by physical barriers such as rivers, built-up areas and highways. Under this hypothesis, multiple physical barriers limit gene flow, resulting in several scattered genetic populations. (c) The urban island hypothesis (Gloor, Bontadina, Hegglin, Deplazes & Breitenmoser, 2001) expects that dispersal may (also) be affected by behavioural barriers, which are most likely to occur at the border of the city where the rural landscape changes into the urban environment. Accordingly, individuals within the city are habituated to manmade structures and human presence, while individuals from the rural surroundings are not and thus face a behavioural barrier to enter the urban area. This predicts two genetic populations resulting from limited gene flow across the city border. We further expect that urban foxes disperse along artificial structures and through built-up areas when crossing the urban matrix.

In order to examine these predictions, we used assignment-based population genetic approaches to identify the location of abrupt genetic discontinuities and resistance-modelling-based landscape genetic approaches to assess the functional connectivity of the landscape. We tested three genetic distance measures to address the performance of different genetic distance measures in model selection and to generate robust results.

2 | MATERIALS AND METHODS

2.1 | Study area, sampling and laboratory procedures

The Berlin metropolitan area (Figure 1a) has 3.5 million inhabitants and covers ~900 km^2. It has been steadily changing during the last century and independent villages and satellite agglomerations were incorporated into the city. Thus, the urban landscape structure is quite heterogeneous, ranging from extremely urbanized areas of dense housing and high proportions of impervious surfaces to districts where forests and lakes represent up to 75% of land cover. The city area includes around 2,500 city parks, some areas of agricultural cultivation, 160 km^2 of forest and several lakes. The countryside around Berlin is characterised by sparse urban agglomerations, agriculture and forest. The landscape transition from the countryside to (sub-)urban areas does not fully correspond to the administrative boundaries between Berlin and Brandenburg as there are several forests, lakes and green areas that reach into the city (Figure 1b). These green spaces and lakes are commonly used as recreational areas.

Between 2010 and 2015, tissue samples from 374 foxes were collected (Figure 1a): Within Berlin, 188 fox carcasses were collected for a municipal disease monitoring program. For each individual, location (street, house number/km, postal code), sex and age category were known. The 186 samples from rural Brandenburg were collected by hunters and made available to the veterinary authorities or the authors of this study. No animal was killed with the aim of providing samples for this study. For 116 Brandenburg samples, the location or the cadastral unit of origin was known, for the remaining 70 individuals, only the nearest village to the harvest site was recorded. In these cases, we chose a random forest location within 2 km of the village recorded as sampling site. No information on sex and age was available for the samples from Brandenburg.

Tissue samples were stored at −20°C or in absolute ethanol. We genotyped the samples using 16 microsatellite loci: DGN3, DGN14, FH2541, REN161A12, REN162B09, REN69B24, V374, V402, V502, Vv-C01.424, Vv-C08.618, Vv-CPH11, Vv-CPH2, Vv-INU055, VVM124, VVM189 (Breen et al., 2001; Mariat, Amigues, & Boscher, 1998; Moore, Brown, & Sacks, 2010; Wandeler & Funk, 2006; Yan et al., 2015). The data are publicly available (see Data Accessibility). Detailed information on laboratory procedures is given in Appendix S1.

2.2 | Population genetic analysis

To assess the suitability of the microsatellites for population genetic analyses, we tested each locus for deviations from Hardy-Weinberg and linkage equilibrium using GENEPOP v.4.7.0 (Rousset, 2008). We also used GENEPOP to calculate F_{IS} values (Weir & Cockerham, 1984). To avoid deviations resulting from Wahlund effects and isolation-by-distance (Frantz, Cellina, Krier, Schley, & Burke, 2009), when analysing the full data set, we subsampled the complete data set to generate 10 data sets consisting of 24 spatially clustered individuals (details in Appendix S2). We tested each set for significant deficiency or excess of heterozygotes and linkage disequilibrium (LD) among loci using the Markov chain method in GENEPOP with 10,000 dememorization steps, 20 batches and 5,000 subsequent iterations. We used the false discovery rate (FDR) to account for multiple tests (Verhoeven, Simonsen, & McIntyre, 2005).

We used two Bayesian-based clustering methods to estimate the number of genetic subpopulations (K), STRUCTURE v. 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) and GENELAND v. 3.3.2 (Guillot, Estoup, Mortier, & Cosson, 2005). Running conditions and specifications are described in Appendix S3. We used Microsatellite analyser MSA 4.05 (Dieringer & Schlötterer, 2003) to calculate observed and expected heterozygosities and the number of alleles across all loci. The level of genetic differentiation between genetic clusters inferred by STRUCTURE and GENELAND was assessed using F_{ST} (Weir & Cockerham, 1984) in SPAGEDI 1.4 (Hardy & Vekemans, 2002); significance was tested with 10,000 permutations of individual genotypes between populations. We analysed the complete data set using the Estimated Effective Migration Surfaces (EEMS) method (Petkova, Novembre, & Stephens, 2016). It uses georeferenced genetic data and can identify locations of abrupt genetic discontinuities (i.e., gene flow barriers) in data sets characterised by isolation-by-distance patterns (details in Appendix S4). We plotted the results for the run with the highest log-likelihood, using the REEMSPLOTS package in R (Petkova et al., 2016).

2.3 | Landscape resistance modelling

Functional connectivity was assessed using RESISTANCEGA 3.1-3 (Peterman, 2018). It calculates pairwise resistance distances between individuals and uses a linear mixed effects model based on genetic algorithms to maximize the fit of resistance surfaces to the

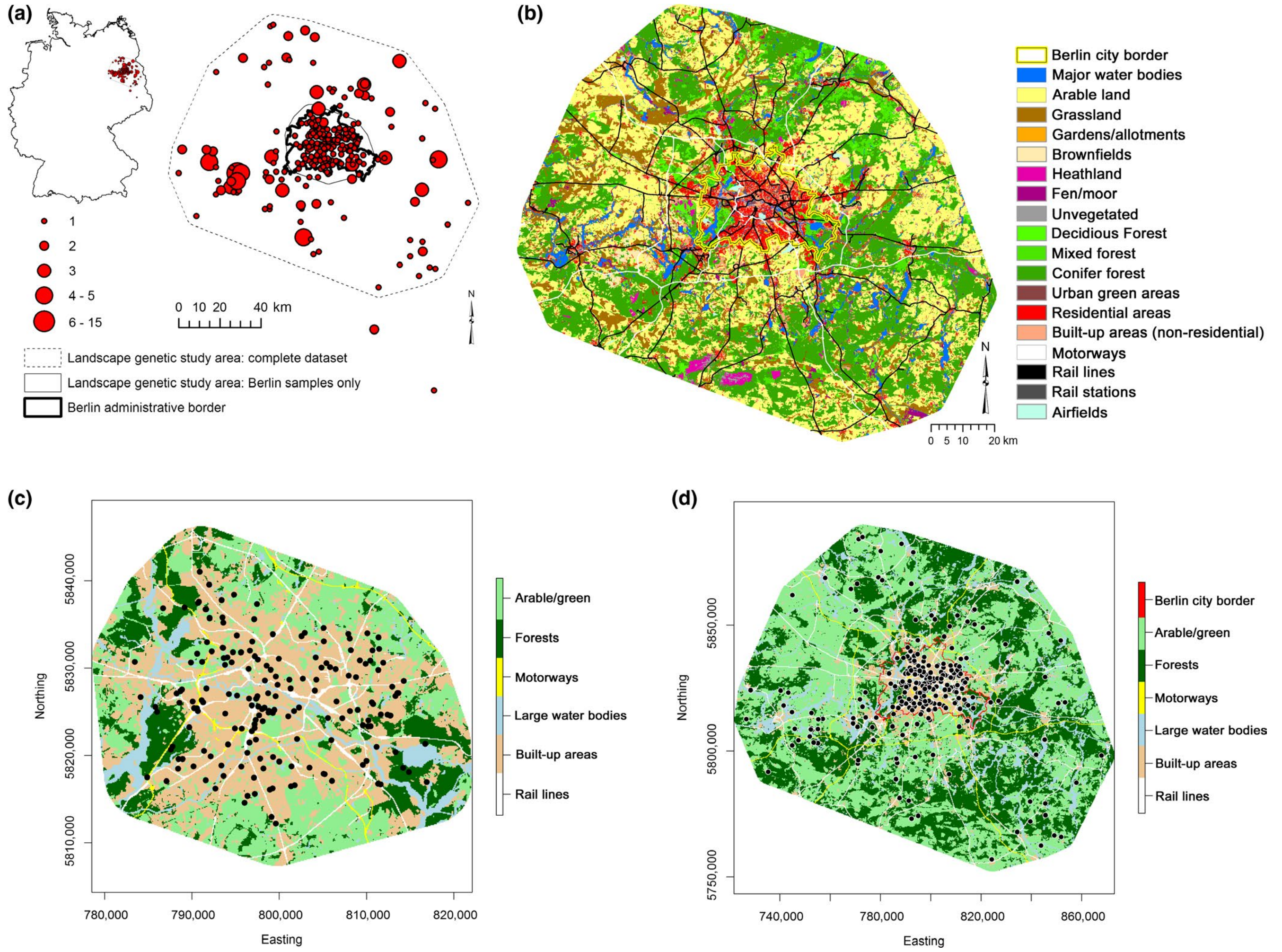


FIGURE 1 Sample distribution and land cover maps of the study area. (a) The location of the study area within Germany and the geographic origin of samples, with size of the circles indicative of the number of samples collected from a locality. The dotted- and thin-lined polygons show the boundaries of the study area used in landscape resistance modelling for the complete data set and the Berlin-only data set, respectively. (b) Land cover map of the landscape genetics study area. (c) Habitat categories considered in the genetic landscape resistance modelling of the city of Berlin, with the black dots showing the location of the 184 samples that were included in the analysis. (d) Habitat categories considered in the genetic landscape resistance modelling of Berlin and the surrounding countryside, with the black dots showing the location of the 286 samples that were included in the analysis. Habitat data were taken from the German authoritative topographic cartographic information system (ATKIS) [Colour figure can be viewed at wileyonlinelibrary.com]

data. The process is based on stochastic search algorithms that solve optimisation problems by mimicking processes of natural selection (Scrucca, 2013). The optimisation process uses log-likelihood as the objective function. Mixed models were fitted using the maximum likelihood population effects (MPLE) parameterization (Clarke, Rothery, & Raybould, 2002) implemented in the R package lme4 (Bates, Mächler, Bolker, & Walker, 2014). A simulation study by Shirk, Landguth, and Cushman, (2018) has shown that this linear-mixed-effects-model-based method had a high accuracy in model selection.

RESISTANCEGA can optimise categorical and continuous resistance surfaces, as well as multiple resistance surfaces simultaneously (Peterman, 2018). All analyses in this work were based on categorical resistance surfaces and the commute-time geographic resistance distance (Kivimäki, Shimbo, & Saerens, 2014), equivalent to circuit-theory-based resistance distances (McRae, Shah, & Mohapatra, 2013). Model fit was assessed with the corrected Akaike information criterion (AIC_c): A specific model was considered a better fit if the difference in AIC_c (ΔAIC_c) to the next model was >2 AIC_c units. To check for convergence, each optimisation run in the study was performed twice for each landscape feature or combination of landscape features. Using the GA.PREP() function, we set the maximum value to be assessed during optimization of categorical resistance surfaces to 500 and retained all other default parameters of the GA.PREP() function.

We performed separate analyses for foxes sampled within Berlin and the full data set including the surrounding countryside. We split the analysis because subtle behavioural patterns within the urban area may be missed in a joint analysis of the complete data set. Also, the greater precision of sampling locations within Berlin allowed a fine-scale analysis of the permeability of the urban environment (see below). Except when mentioned, ARCMAP v.10.3 (ESRI Inc) was used to prepare resistance surfaces. Potential movements of individuals at the edge of the study area can be artificially constrained by the proximity to the boundary (Koen, Garroway, Wilson, & Bowman, 2010). The extent of the study areas was therefore obtained by plotting a minimum convex polygon around the sampling locations. Then, based on dispersal distances obtained by capture-mark-recapture methods (Harris & Trewhella, 1988; Trewhella & Harris, 1990) we added a 5 km buffer around this feature.

Landscape classification was based on the German topographic cartographic information system ATKIS (Figure 1b, Gruenreich, 1992). Seven landscape categories, hereafter called environmental predictors, were considered to potentially influence gene flow (Figure 1c–d, Figure S1). (a) *arable/green*: all types of arable land and grassland, fallow land, allotments, airports, public parks, cemeteries and bare soils; (b) *built-up areas*: residential, industrial and commercial areas; (c) the *city border* of Berlin (or variations thereof; see below); (d) *forests*: irrespective of their composition; (e) *motorways*: with tunnelled sections within the city not considered; (f) *railways*: including major stations but excluding tramways and (g) larger *water bodies*: including lakes and major rivers (which do not overlap with other shapes), excluding small streams, creeks and underground canals (line elements that overlap other shapes). For the initial analyses within the city area only, we pooled all arable land, green and forests into a single (h) *all vegetation* layer (Figure 1c; Figure S2). To distinguish the actual landscape from environmental predictors used for functional connectivity analysis, environmental predictors appear in italics throughout the text.

In addition to ATKIS, we used data from the 2012 Copernicus Urban Atlas (https://land.copernicus.eu/) that uses high-resolution remote sensing data to provide detailed land cover information of larger European urban areas and their hinterland. It comprised 27 different land cover types (Table S1), of which six, classified under *urban fabric*, give an indication of the degree of imperviousness of land cover (Montero, Van Wolvelaer, & Garzón, 2014). We used the following (nonoverlapping) categories of the Urban Atlas to subdivide the *built-up area* layer within the city (see Figure S3): (a) *Continuous urban fabric* (sealing level (S.L.) >80%), (b) *discontinuous dense urban fabric* (S.L. 50%–80%), (c) *discontinuous medium dense urban fabric* (S.L. 30%–50%), (d) *industrial, commercial, public, military and private units* and (e) a pooled layer consisting of *discontinuous low density urban fabric* (S.L. 10%–30%), *discontinuous very low density urban fabric* (S.L. <10%) and all the remaining built-up areas not covered by the previous categories.

We converted all layers into grids. Not considering cells without data, each cell in the initial grid had a value of zero or one, depending on whether it contained a feature under consideration. For linear predictors and *water bodies* we used a priority rule, meaning that every grid cell containing a linear predictor was coded as belonging to that predictor, independently of the proportion of the cell it covered. For shape predictors, we used a majority rule, with the cell being attributed to the single predictor with the largest area within the cell. Grid cell size was set to 250 × 250 m, giving rise to 233,798 grid cells without 'no data' cells, when ignoring three geographic outliers (Figure 1a). Since we only considered one animal per grid cell, this resulted in 286 individuals being included in the analysis. Given the ecology of the species and the occasional lack of precision of the location information, we considered this to be an adequate compromise between computation time and spatial resolution. When focusing on the individuals in Berlin only, grid cell size was set to 100 × 100 m, given the higher accuracy of the sampling location and the smaller size of the study area. This resulted in 125,728 grid cells without "no data" cells, and 184 individuals being analysed.

2.4 | Genetic distance measures

Interindividual genetic distance measures are not equally accurate in model selection, especially when faced with weaker genetic structure (see Shirk et al., 2017). We therefore compared the performance of three measures: (a) genetic distances based on Factorial Correspondence Analysis (FCA), an eigenvector-based multivariate analysis closely related to principal component analysis (PCA); (b) Nei's genetic distance applied to individuals (D_{Nei}, Nei & Takezaki, 1983), as used by Beninde et al. (2016) and (c) the proportion of shared alleles between two individuals averaged over loci (D_{PS}, Bowcock et al., 1994), that is frequently used in landscape resistance modelling (Landguth et al., 2010; Trumbo, Spear, Baumsteiger, & Storfer, 2013). FCA clusters variance between loci into composite gradients. It accentuates differences between individuals better than measures that weight all loci equally. The latter includes D_{PS}, which uses the number of direct differences between genotypes. D_{Nei} considers allele frequencies when calculating genetic distances and ranges from 0 for identical genotypes to 1 when genotypes are completely dissimilar.

We used GENETIX v. 4.05.2 (Belkhir, 2004) to perform an FCA on a multiple contingency table of the genetic data and used the first 10 FCA axes as a compromise between model accuracy and noise generation (Shirk et al., 2017). We calculated an Euclidean distance matrix for all individuals from their values on each FCA axis using the R package Ecodist (Goslee & Urban, 2007) and refer to this distance measure as "FCA". We used the R packages Alleles in Space (Miller, 2005) to calculate D_{Nei} and Adegenet (Jombart, 2008) to calculate D_{PS}.

2.5 | Optimisation of resistance surfaces: Single categorical environmental predictors

We first used the SS _ OPTIM() command in RESISTANCEGA to optimise the resistance of single categorical environmental predictors and test model selection performance of the genetic distance measures. In order to complete these analyses within a reasonable time, we limited initial tests to the seven ATKIS predictors (five for Berlin only). We performed a (pseudo-)bootstrap procedure using the RESIST.BOOT() command, which subsamples individuals and resistance matrices without replacement at each iteration, refits the MLPE model to different resistance distance matrices and recalculates AIC$_c$ scores. We sampled 75% of the observations at each iteration. This was done in order to assess the relative support of each optimised resistance surface and the robustness of the model selection results given different sample combinations. For each genetic distance measure, we assessed model fit based on the differences between corrected Akaike information criterion (ΔAIC$_c$) values. When comparing genetic distance measures, the measure that gave rise to the highest marginal R^2 values (while generating biologically meaningful results) was considered the most adequate.

2.6 | Multiple resistance surfaces

After optimising individual categorical resistance surfaces, the relevant variables must be combined into a composite resistance surface. This is necessary to test whether models with several landscape features are better supported than models with single landscape features and, ultimately, to gain an understanding of the functional connectivity of the entire landscape (Khimoun et al., 2017; Ruiz-Lopez et al., 2016).

2.7 | Multiple resistance surfaces: Automatically combining categorical predictors

We used the samples from Berlin, the best genetic distance measure and the five ATKIS categories to compare two approaches that combine categorical predictors into a single surface. Firstly, we used RESISTANCEGA's ALL _ COMB() wrapper function which automatically combines and optimises all possible combinations of the five categorical predictors and runs the RESIST.BOOT() command to conduct a bootstrap analysis. However, the MS _OPTIM() command gives different resistance values to a linear feature depending on which other feature it overlaps with, which may lead to erroneous conclusions (Section 3).

2.8 | Multiple resistance surfaces: Single-surface optimisation for combining categorical predictors

We therefore also tested a second approach for combining categorical predictors into a single surface: Rather than letting RESISTANCEGA automatically combine different surfaces, we applied the single-surface optimisation (SS _OPTIM()) procedure to resistance grids containing multiple environmental predictors, i.e. each grid contained *N* categorical predictors and each cell in the grid had a value ranging from zero to *N*, depending on whether it was classified as one of the *N* predictors or whether it was classified as *matrix*, i.e., the remaining uniform study area not containing the features under investigation. We will refer to these grids as "multicategorical" surfaces (to differentiate them from composite surfaces obtained using ALL _ COMB()). The principle underlying the multicategorical models is to add individual predictors based on model support (AIC$_c$ values) but to only retain a new predictor if its addition improved support (ΔAIC$_c$ > 2 after a RESIST.BOOT() bootstrap analysis). Individual predictors whose model support was ΔAIC$_c$ < 2 with *distance* were not considered. The optimisation for each feature or combination of features was performed twice and only included the distance matrix from the optimisation run with the lowest AIC$_c$ value in bootstrap analysis. We will refer to this as the "stepwise optimisation" procedure.

2.9 | Multiple resistance surfaces: Dealing with overlapping linear features

In order to assess the effect of the overlap of linear predictors, we considered all possible priority combinations of predictors. We tested, for example, individual surfaces where linear predictor 1 took precedence over linear predictor 2 at points of overlap and vice versa. We also tested the support of a surface where all points of overlap between linear features were classified as a distinct feature. In each case, the combination with the highest model support after bootstrapping was retained for further analysis.

2.10 | Multiple resistance surfaces: Effect of initial cell values of multicategorical surfaces

Preliminary exploratory analyses suggested that in the stepwise optimisation, the initial cell values of a predictor influenced the optimised resistance value for the predictors (and hence model support). We therefore coded individual predictors relative to their resistance/permeability inferred in the initial individual analysis. For example, in order to obtain the highest model support when manually combining two different categorical predictors in a single grid, a predictor inferred to be permeable had to be given a grid value of zero, a predictor resisting gene flow a grid value of two and all other cells a value of one. In order to test more formally whether the optimised resistance values were sensitive to the starting values of the input surface, we took multicategorical surfaces with different combinations of predictors that were retained in the stepwise optimisation procedure and inverted the values of the input surface. We performed a total of four independent optimisation runs for each initial and inverted surface.

2.11 | Multiple resistance surfaces: Optimising multicategorical surfaces of Berlin

After these initial tests based on ATKIS categories *all vegetation*, *built-up areas*, *water bodies*, *motorways*, *railways*, we refined the composite Berlin model further. We performed single-surface optimisation for both the *forest* and *arable/green* layers (which had been previously pooled in *all vegetation*) as well as the five Urban Atlas categories of *built-up areas* to test model support of each individual layer. We then followed a stepwise optimisation procedure to generate a multicategorical surface. If the difference in model support between individual predictors was ΔAIC$_c$ < 2, we added both predictors individually and jointly to the previous multicategorical model and only retained the model with the highest support.

2.12 | Multiple resistance surfaces: Optimising multicategorical surfaces of the whole study area

We also used a stepwise procedure to generate a multicategorical surface for the whole Berlin/Brandenburg study area. To gain a more detailed assessment of the interface between the city and the surrounding countryside as a possible gene flow barrier, we created a concave hull of the administrative city border using the ConcaveHull plug-in for QGIS (QGIS Development Team, 2018). We then drew 1, 2, 3, 4 and 5 km buffers around the concave hull and used the SS _ OPTIM() command in RESISTANCEGA to separately optimise the resistance of all five inner and outer borders (Figure S4). We then used the boundary model with the highest support together with the six remaining ATKIS predictors to identify the best-supported multicategorical surface. Again, we performed the RESIST.BOOT() bootstrap analysis for each optimisation run, to circumvent potential problems with imprecise locations of individuals sampled in Brandenburg.

The best-supported multicategorical resistance surfaces for the Berlin and Berlin/Brandenburg data sets were used to predict movement/gene flow patterns across both study areas using CIRCUITSCAPE v.4.0.5 (McRae, 2006). Animal movement paths were inferred between all pairs of sample location as well as between 200 random locations generated for both data sets using ARCMAP v.10.3 and located along the border of the study areas.

3 | RESULTS

After correction for multiple tests, locus V502 deviated from Hardy-Weinberg equilibrium (HWE) in five out of 10 subsampled data sets (Table S2), its F_{IS} values ranging between 0.40–0.53 in these five data sets. The locus was thus excluded from further analyses. No other locus showed systematic deviation from HWE. Some loci were in linkage disequilibrium in some subsampled data sets, but no pair deviated more than once (Table S3). We therefore performed further population genetic analyses with all loci except V502.

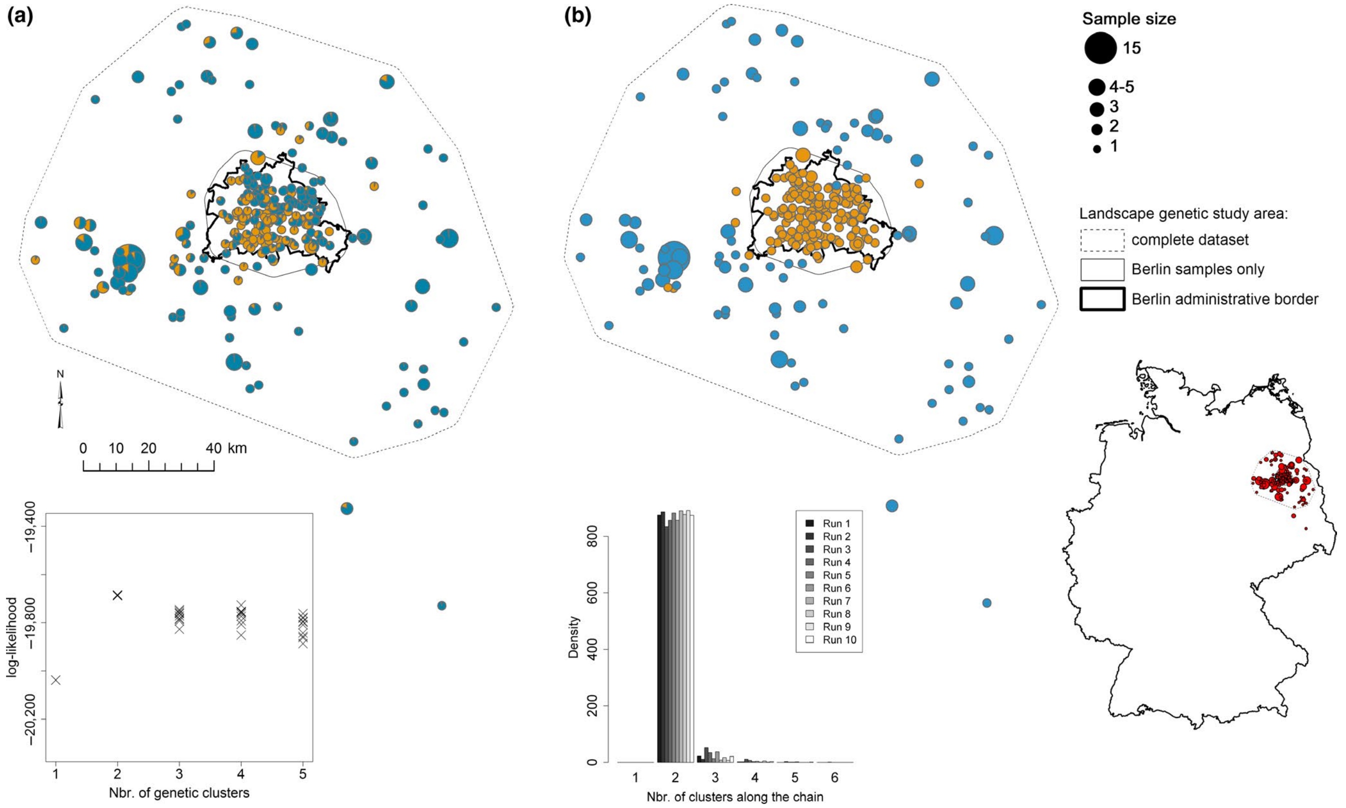
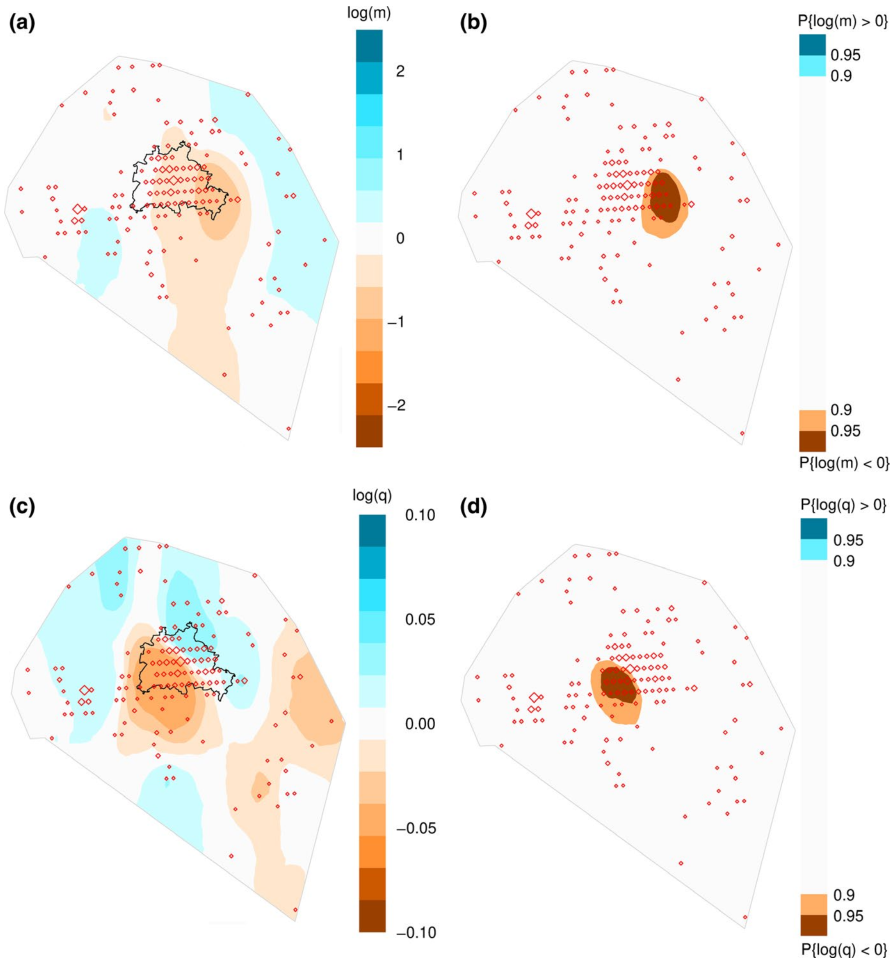


FIGURE 2 Geographic distribution of the population genetic clusters. (a) STRUCTURE results: plot of the number of clusters against their estimated log-likelihood (bottom) and geographic representation of the $K = 2$ assignment analysis (top). The pattern of the pie chart indicates the assignment probabilities averaged across all individuals sampled in the same location, with the two different colours representing membership of different clusters and the size indicating the number of collected samples from one locality. (b) GENELAND results: plot of the number of clusters inferred by the ten initial GENELAND runs (bottom) and geographic representation of the modal assignment to one of the two clusters, i.e., the pattern of the pie chart indicates the proportion of individuals from a locality assigned to one of the two clusters (top). Bottom right insert shows the location of the study area within Germany [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 3 EEMS-estimated effective migration and diversity rates. (a) Interpolated surface of the posterior mean migration rates *m* (on a $\log_{10}$ scale) depicting deviations from continuous gene flow. Negative values in red indicate areas of reduced migration rates, whereas positive values in blue indicate higher-than-expected migration rates. (b) Plot highlighting regions where the effective migration rates are significantly higher (highlighted in blue) or lower (highlighted in orange) than the overall average rate. (c) Interpolated surface of the posterior mean diversity rates *q* (on a $\log_{10}$ scale) depicting effective diversity across the study area. Diversity rates *q* describe the genetic dissimilarity between distinct individuals from the same deme. Negative values in red indicate areas of diversity, whereas positive values in blue indicate higher-than-expected diversity. (d) Plot highlighting regions where the effective diversity rates are significantly higher (highlighted in blue) or lower (highlighted in orange) than the overall average rate. A total of 1,000 demes were used in the analyses [Colour figure can be viewed at wileyonlinelibrary.com]



3.1 | Population structure

The log-likelihood values inferred by STRUCTURE provided clear support for the presence of two genetic clusters (Figure 2a). The corresponding clusters approximately consisted of (a) samples collected in the centre, west and south of Berlin and (b) samples from all rural localities and north-eastern Berlin, yet their precise geographic distribution was not clear-cut (Figure 2a; Figure S5). The location of the genetic discontinuity identified by STRUCTURE approximately corresponds to the course of the rivers Spree and Havel (Figure S5). GENELAND also inferred $K = 2$ as the most likely number of clusters in each of the 10 initial runs (Figure 2b). The samples assigned to one cluster almost all originated from within the Berlin city border, whereas the second cluster contained samples almost exclusively collected in the countryside (Figure 2b), i.e., GENELAND identified an urban and a rural cluster, with the boundary quite accurately corresponding to the administrative city border. The EEMS contour plot of effective migration rates identified a band of (slightly) reduced long-distance migration rates that covered most of the city, but also extended to the south of the study area (Figure 3a). In the east of Berlin, migration rates were significantly lower than the overall average rate (Figure 3b).

Independently of the clustering method, the more urban cluster had reduced genetic diversity compared to the rural cluster (Table S4) and the clusters were significantly differentiated from each other. Differentiation between both STRUCTURE clusters ($F_{ST} = 0.026$; $p < .0001$) was higher than between the two GENELAND clusters ($F_{ST} = 0.011$; $p < .0001$). The EEMS contour plot of effective diversity illustrated that in southwest Berlin effective diversity rates were significantly lower than the overall average rate (Figure 3c, d).

3.2 | Optimisation of resistance surfaces: Single categorical environmental predictors

When considering the five ATKIS environmental predictors, the results obtained after bootstrapping (Table 1) were qualitatively similar to initial model results (Table S5). The three genetic distance measures did not converge on the same results in the model selection process (Table 1). In the analyses using D_{Nei} and D_{PS}, *motorways* was always identified as the most significant factor facilitating gene flow, with *railways* ranked as second best model (also facilitating gene flow) and all other models (except one: *water bodies* in one run using the D_{PS} genetic measure) having a difference in $\Delta AIC_c < 2$ with the *distance* model. In the FCA, the difference between the *distance* model and all five predictors was large ($\Delta AIC_c > 6.2$), with the ranking of the five models remaining identical between the two independent optimisation runs (Table 1). The *water bodies* model that best explained gene flow with the FCA measure (*water bodies* being a strong gene flow barrier) had an average marginal R^2 of 0.289, a substantially higher model fit than the best model's average marginal R^2 of 0.057 and 0.054 for D_{Nei} and D_{PS}, respectively. We therefore used FCA for further analyses.

3.3 | Multiple resistance surfaces: Automatically combining categorical predictors

When considering the prebootstrapping results of the optimisation of all possible combinations of the five single ATKIS environmental predictors (ALL _ COMB() function, Table S6), only three combinations had a $\Delta\mathrm{AIC_c} > 2$ below the best single-feature model (*water bodies*). After bootstrapping, 11 combinations had a $\Delta\mathrm{AIC_c} > 2$ below the *water bodies*-only model, with the best model containing *railways* and *water bodies* (resistance values: *railways*: 1, *matrix*: 185, overlap *water bodies/railways*: 250, *water bodies*: 434; Table S7; Figure S6). When simultaneously optimising and overlaying different categorical resistance surfaces that included linear features, RESISTANCEGA gave different resistance values to a linear feature depending on which feature it overlapped with. For example, in the model that included all five environmental predictors, RESISTANCEGA optimised the resistance value of 24 different categorical features (Figure S7).

3.4 | Multiple resistance surfaces: Single-surface optimisation for combining categorical predictors & dealing with overlapping linear features

The two best-supported models in the FCA-based analysis of individual features were *water bodies* and *railways*. When combining these two predictors in a single-surface optimisation, the highest model support was obtained (after bootstrapping) when giving *water bodies* precedence over *railways* in the resistance grid (when *water bodies* overlap with *railways*, the cell is classified as water body; Table S8). When adding the next best-supported *motorways* model to the single-surface analysis, the highest model support was obtained when *water bodies* took precedence over *motorways* and *motorways* took precedence over *railways* (*water bodies* > *motorways* > railways; Table S9). After bootstrapping, the three best "overlap" models had almost identical model support ($\Delta\mathrm{AIC_c} < 2$; Table S9). *Water bodies* always strongly impeded gene flow, while *railways* and *motorways* conducted gene flow. When adding *all vegetation* (and hence *built-up areas*) to each of these three overlap models in a single-surface analysis, the model with *water bodies* > *motorways* > railways was again the best-supported model after bootstrapping (Table S10). In summary, when only considering the ATKIS data, the best permeability model for Berlin included all five tested features (Table 2; Figure S8). *Water bodies* strongly resisted gene flow (resistance: 1574), *railways* (resistance: 1) and *motorways* (resistance: 4) enhanced gene flow. *Built-up areas* (resistance: 291) were more permeable than *all vegetation* (resistance: 494). The following analyses were based on the *water bodies* > *motorways* > railways overlap model.

3.5 | Multiple resistance surfaces: Effect of initial cell values of multicategorical surfaces

Multicategorical models whose starting cell values had been inverted gave rise to different optimised resistance values for the predictors and had a lower model support than the noninverted original multicategorical surfaces (Table S11).

3.6 | Multiple resistance surfaces: Optimising multicategorical surfaces of Berlin

When repeating single-feature optimisations but splitting the *all vegetation* predictor into the two predictors *forest* and *arable/green* and the *built-up areas* into the five Urban Atlas categories, *water bodies*, *railways* and *motorways* were still the individual features with the highest model support (Table 2), with *arable/green* generating a better model support than the *all vegetation* model (Table 2). Similarly, two Urban Atlas land cover categories (*sealing levels* [*S.L.*] of 30%–50% and >80%) had higher model support than the predictor including all built-up areas (Table 2). The fourth-best (*arable/green*) and the fifth-best (*S.L.* 50%–80%) individual models had similar model support ($\Delta\mathrm{AIC_c} = 0.5$; Table 2). The resistance value inferred for each single predictor is given in Table S12. A better-supported model was obtained when adding *S.L.* 30%–50% to the *water bodies*, *railways*, *motorways* ("*mrw*") model than when adding the *arable/green* predictor or both *arable/green* and *S.L.* 30%–50% to the *mrw* model (Table 2). Adding further single-feature predictors to the single-feature optimisation procedure in order of decreasing support (and testing all possible combinations when $\Delta\mathrm{AIC_c} < 2$ between two individual predictors) resulted in three multicategorical models having comparable support (Table 2). The overall best model (Figure 4) included *railways* (inferred resistance value: 1), *motorways* (resistance: 2), *S.L.* 30%–50% (resistance 8), *S.L.* 50%–80% (resistance: 103), *S.L.*>80% (resistance: 469), water bodies (resistance: 784) as well as the remaining *matrix* (resistance: 282). Despite differences in the resistance surface values between the models, the CIRCUITSCAPE current maps for the best supported model and the model with the fewest predictors were very similar, both suggesting that gene flow within the city of Berlin mostly occurred along linear landscape elements (*railways* and *motorways*, Figure 4).

3.7 | Multiple resistance surfaces: Optimising multicategorical surfaces of the whole study area

All *city border* models and obtained better support than the distance only model and inferred the city border to resist gene flow (Table

TABLE 1 Boostrap results of the single-predictor RESISTANCEGA analysis for the city of Berlin

	Run 1					Run 2				
Predictor	**avg. AIC$_c$**	***k***	**ΔAIC$_c$**	**avg.weight**	**avg.mR^2**	**avg. AIC$_c$**	***k***	**ΔAIC$_c$**	**avg.weight**	**avg.mR^2**
(a) Nei's genetic distance (D_{Nei})										
Motorways	−21,765.0	3	0	0.977	0.035	−21,770.8	3	0	0.977	0.036
Railways	−21,750.0	3	15.0	0.016	0.057	−21,755.5	3	15.3	0.016	0.057
Built-up areas	−21,747.9	3	17.1	0.002	0.017	−21,753.4	3	17.4	0.002	0.017
Water bodies	−21,747.9	3	17.1	0.002	0.022	−21,753.3	2	17.5	0.002	0.018
Distance	−21,747.8	2	17.2	0.002	0.018	−21,753.3	3	17.5	0.002	0.022
All vegetation	−21,747.7	3	17.3	0.001	0.018	−21,753.2	3	17.6	0.001	0.018
(b) Proportion of shared alleles (D_{PS})										
Motorways	−21,744.2	3	0	0.418	0.025	−21,761.1	3	0	0.946	0.034
Railways	−21,741.1	3	3.1	0.228	0.054	−21,748.9	3	12.2	0.038	0.054
Water bodies	−21,740.6	3	3.6	0.258	0.051	−21,746.5	3	14.6	0.004	0.017
Built-up areas	−21,738.7	3	5.5	0.036	0.017	−21,746.3	2	14.8	0.004	0.018
Distance	−21,738.5	2	5.7	0.031	0.018	−21,746.2	3	14.9	0.004	0.017
All vegetation	−21,738.4	3	5.8	0.029	0.017	−21,746.1	3	15.0	0.004	0.020
(c) Ten-axes-based factorial correspondence analysis (FCA)										
Water bodies	130,757.2	3	0	0.701	0.289	130,743.3	3	0	0.707	0.289
Railways	130,771.3	3	14.1	0.222	0.057	130,759.2	3	15.9	0.206	0.057
Motorways	130,779.9	3	22.7	0.076	0.014	130,766.9	3	23.6	0.087	0.014
All vegetation	130,800.7	3	43.5	0.001	0.030	130,787.4	3	44.1	<0.001	0.027
Built-up areas	130,803.4	3	46.5	<0.001	0.029	130,790.3	3	47.0	<0.001	0.029
Distance	130,810.0	2	52.8	<0.001	0.007	130,796.9	2	53.6	<0.001	0.007

Notes: Three different genetic distance measures (a–c) and five environmental predictors from the German authoritative topographic cartographic information system (ATKIS) were compared (Section 2). The initial model results are presented in Table S5. To check for convergence, each optimisation was performed twice for each landscape feature (Run 1 & Run 2). Avg. AIC$_c$, average of the AICc values obtained for each model in 1,000 bootstrap iterations; ***k***, number of parameters; **ΔAIC$_c$**, difference in the avg. AIC$_c$ values between the best supported model (lowest avg. AIC$_c$) and each subsequent model; Avg.weight, average of the AIC$_c$ weights obtained for each model in 1,000 bootstrap iterations; Avg.mR^2, average marginal R^2 of 1,000 bootstrap iterations. Predictors are sorted according to increasing avg.AIC$_c$ values

S13). The best-supported model (the city border converted into a concave hull) had a marginal R^2 of 0.42. The *city border concave* was also the most significant single predictor influencing gene flow when considering all other predictors (Table 3; Figure S9). Considering the bootstrapping results of the FCA-based genetic distance only, five of the six remaining single-feature models better explained gene flow than the distance only model (the exception being *motorways*; Table 3). Forest and arable/green were the only environmental features inferred to facilitate gene flow (Table S14). Again, the three genetic distance measures did not converge on the same results in the model selection process (Table 3), with the support of the *city border* model in particular changing with the genetic distance measure considered. Also, the marginal R^2 values obtained with D_{Nei} and and D_{PS} were considerably lower than those obtained with the FCA-based measure (Table 3).

When performing a stepwise procedure to create a multicategorical surface, two multicategorical models had almost identical support. The overall best-supported model (after bootstrapping) contained *city border concave*, *built-up areas*, *railways* and *water bodies* (Table 4), where *water bodies* took precedence over *railways* (Table S15). While *railways* (resistance: 1) and the remaining habitat *matrix* (resistance: 2) enhanced gene flow, *city border concave* (resistance: 498) and *water bodies* (resistance: 70) provided a greater resistance than *built-up areas* (resistance: 6). The second-best model (with almost identical support) had the same predictors (with similar resistance values) but did not include *railways*. Considering both models, the CIRCUITSCAPE maps did not show a clearly-defined corridor network in the Brandenburg countryside (Figure S9).

4 | DISCUSSION

In the present work, we aimed to assess the importance of physical and behavioural dispersal barriers to drive population and landscape genetic structure of the red fox across the Berlin metropolitan area. We found support for the fragmentation hypothesis with major water bodies and densely built-up areas resisting gene flow. Contrary to our prediction, however, these barriers did not create several scattered populations across the city, possibly

TABLE 2 Results of the multicategorical functional connectivity analysis for the city of Berlin

Predictors	avg.AIC$_c$	*k*	ΔAIC$_c$	avg.weight	avg.mR^2
Water*Railways*Motorways*S.L.30%–50%*S.L.50%–80%*S.L. > 80%	130,726.1	8	0	0.194	0.169
Water*Railways*Motorways*S.L.30%–50%*S.L.50%–80%*S.L. > 80%*Industry	130,726.7	9	0.6	0.244	0.199
Water bodies*Railways*Motorways*S.L.30%–50%*S.L. > 80%	130,727.4	7	1.3	0.153	0.188
Water bodies*Railways*Motorways*S.L.30%–50%	130,730.2	6	4.1	0.044	0.168
Water*Railways*Motorways*S.L.30%–50%*S.L. > 80% *Industry	130,730.6	8	4.5	0.115	0.184
Water*Railways*Motorways*S.L.30%–50%*S.L.50%–80%*S.L. > 80% *Remaining built-up	130,731.8	9	5.7	0.072	0.152
Water*Railways*Motorways*S.L.30%–50%*S.L. > 80% *Remaining built-up	130,731.9	8	5.8	0.030	0.150
Water*Railways*Motorways*All vegetation*Built-up areas	130,736.5	6	10.4	0.031	0.157
Water bodies*Railways *Motorways*Arable/green	130,738.2	6	12.1	0.039	0.113
Water bodies*Railways*Motorways*Arable/green*S.L.30%–50%	130,738.3	7	12.2	0.058	0.220
Water bodies*Railways *Motorways	130,741.7	5	15.6	0.011	0.195
Water bodies*Railways	130,749.5	4	23.3	0.005	0.087
Water bodies	130,769.1	3	43.0	0	0.293
Railways	130,784.0	3	57.9	0	0.057
Motorways	130,792.9	3	66.8	0.003	0.014
Arable/Green	130,800.5	3	74.3	0.001	0.049
S.L.30%–50%	130,801.6	3	75.4	0	0.017
S.L. > 80%	130,809.1	3	83.0	0	0.051
All vegetation	130,813.7	3	87.6	0	0.030
S.L.50%–80%	130,815.5	3	89.4	0	0.038
Built up areas	130,816.5	3	90.4	0	0.029
Industry	130,817.4	3	91.3	0	0.030
Remaining built-up	130,820.8	3	94.7	0	0.023
Forest	130,822.9	3	96.8	0	0.009
Distance	130,823.1	2	97.0	0	0.007

Notes: The best-supported multicategorical surfaces combining different environmental predictors were obtained using a stepwise procedure: Individual predictors were added based on model support (corrected Akaike information criterion values, AIC$_c$), but only retained if their addition improved support of the multicategorical model (ΔAIC$_c$ > 2). Presented here are the bootstrapping results based on two optimisation runs (Table S12) that were performed for each (combination of) landscape features. avg. AIC$_c$, average of the AIC$_c$ values obtained for each model in 1,000 bootstrap iterations; *k*, number of parameters; ΔAIC$_c$, difference in the avg; AIC$_c$, values between the best supported model (lowest avg.AIC$_c$) and each subsequent model; avg.weight, average of the AIC$_c$ weights obtained for each model in 1,000 bootstrap iterations; avg.mR^2, average marginal R^2 of 1,000 bootstrap iterations. Predictors are sorted according to increasing avg.AIC$_c$ values. S.L., sealing level

because motorways and railways served as movement corridors. We also found support for the urban island hypothesis and inferred limited gene flow across the city border, indicating an effect of behavioural barriers. Urban foxes further made use of artificial structures when dispersing through the urban matrix. Our results may thus suggest a hierarchy of drivers of genetic structure with a general behavioural effect and impediment through physical barriers underneath. However, the specifics of our results also suggest that genetic structure was relatively weak and, therefore, dispersal rates still high.

4.1 | Population genetic structure and gene flow

The genetic clustering algorithms both inferred $K = 2$ as the mostly likely number of subpopulations, yet they differed in the spatial distribution of the clusters. For GENELAND, the cluster boundary closely coincided with the administrative city border, whereas for STRUCTURE the urban cluster mostly excluded the north and northeast of the city. The location of the STRUCTURE-inferred genetic discontinuity approximately corresponded to the course of the rivers Spree and Havel (Figure S5). EEMS also identified reduced

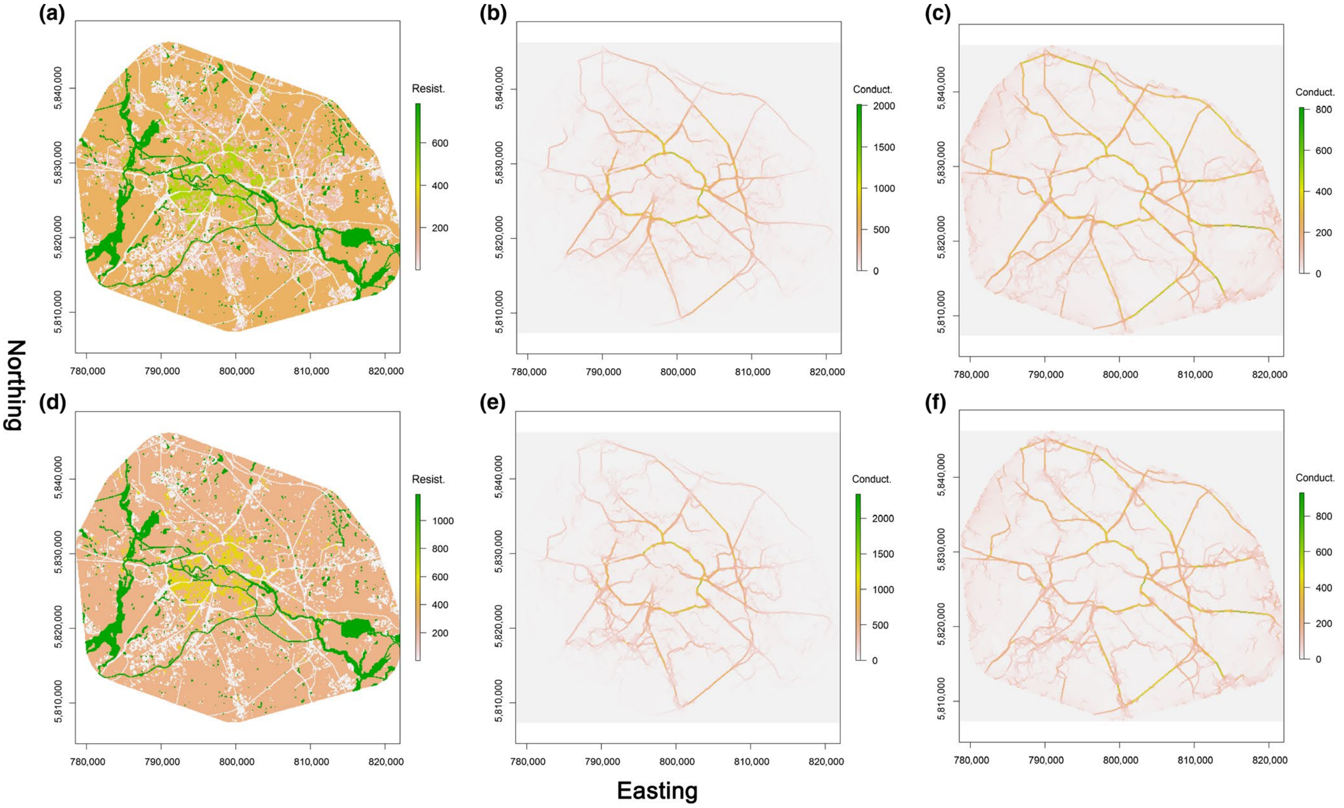


FIGURE 4 Cartographic representation of results from genetics-based resistance modelling for foxes in the city of Berlin. (a) Optimised resistance surface of the overall best multicategorical model and the corresponding Circuitscape connectivity map showing conductance to gene flow based on (b) the sample locations and (c) sampling locations simulated at the edge of the study area. (d) Optimised resistance surface of the best-supported multicategorical model with fewest predictors and the corresponding Circuitscape connectivity map showing conductance to gene flow based on (e) the sample locations and (f) sampling locations simulated at the edge of the study area [Colour figure can be viewed at wileyonlinelibrary.com]

migration (broadly) around the city of Berlin, but especially in East Berlin. Despite discrepancies, all three population genetic methods inferred the presence of a cluster located within the confines of the city. Furthermore, the landscape resistance modelling identified (a concave hull of) the administrative city border as the main barrier to fox dispersal in the study area (Figure S9). Finally, the F_{ST}-based approach and the EEMS method confirmed reduced genetic diversity within (parts of) the city compared to the surrounding countryside. Our results therefore provided general support for a genetic differentiation between urban and rural areas, i.e., the urban island hypothesis.

While the three population genetic methods inferred the presence of an urban island, they differed in its proposed location and composition. Different solutions for the partitioning of a data set may result from differences in the assumptions and algorithms underlying the statistical methods (Guillot et al., 2009) and the way they deal with weak or hierarchical genetic structure (Frantz et al., 2006; Puechmaille, 2016; Rowe & Beebee, 2007) as well as with deviations from random mating that are not due to physical barriers (e.g., isolation-by-distance, presence of relatives, Rodríguez-Ramilo & Wang, 2012). As all three methods inferred a 'circular' cluster in the centre of the sampling distribution and the diversity within the city was reduced, it appears unlikely that the partitioning was an artefact of an isolation-by-distance pattern (Frantz et al. 2009). Perhaps the most likely explanation for the observed outcome is that population genetic structure is weak because of high dispersal rates in our vagile study species. A simulation study suggested that STRUCTURE was efficient at inferring the correct number of genetic clusters even at lower levels of genetic differentiation (i.e., F_{ST} = 0.02–0.03), but this was not necessarily the case for its accuracy in assigning individuals to these clusters (Latch, Dharmarajan, Glaubitz, & Rhodes, 2006). While, by definition, GENELAND infers abrupt genetic discontinuities, the deviation from IBD inferred by EEMS also appeared to be relatively slight (Figure 3). We therefore conclude that our results provided evidence for genetic differentiation between urban and rural foxes, but that dispersal between urban and rural areas was ongoing.

4.2 | Performance of genetic distance measures

While RESISTANCEGA offers high potential to gain a species-specific understanding of the functional connectivity of the landscape, careful consideration of some technical aspects seems necessary. In the present study, the fit of a model testing single categorical environmental predictors and its rank relative to other predictors clearly differed between genetic distance measures. In the simulation study by Shirk et al. (2017) most metrics performed equally

TABLE 3 Boostrap results of the single-predictor RESISTANCEGA analysis for the complete data set

	Run 1					Run 2				
Predictor	**avg. AIC_c**	***k***	**ΔAIC_c**	**avg.weight**	**avg.mR^2**	**avg. AIC_c**	***k***	**ΔAIC_c**	**avg.weight**	**avg.mR^2**
(a) Nei's genetic distance (D_{Nei})										
Built-up areas	310,883.2	3	0	0.838	0.008	310,901.4	3	0	0.824	0.008
Forests	310,890.0	3	6.8	0.156	0.014	310,907.9	3	6.5	0.171	0.014
Arable/green	310,904.2	3	21.0	0.003	0.009	310,922.6	3	21.2	0.004	0.009
City border concave	310,929.6	3	46.4	0.003	0.016	310,947.6	3	46.2	0.002	0.017
Water bodies	310,938.6	3	55.4	<0.001	0.014	310,957.3	3	55.9	<0.001	0.014
Railways	310,942.3	3	59.1	<0.001	0.010	310,960.7	3	59.3	<0.001	0.010
Motorways	310,955.9	3	72.7	<0.001	0.028	310,974.2	3	72.8	<0.001	0.028
Distance	310,956.8	2	73.6	<0.001	0.012	310,975.3	2	73.9	<0.001	0.011
(b) Proportion of shared alleles (D_{PS})										
Built-up areas	310,892.3	3	0	0.956	0.007	310,921.3	3	0	0.951	0.007
Forests	310,902.5	3	10.2	0.039	0.011	310,931.4	3	10.1	0.044	0.011
Arable/green	310,909.8	3	17.5	0.004	0.008	310,939.1	3	17.8	0.004	0.008
City border concave	310,937.3	3	45.0	0.001	0.014	310,967.0	3	45.7	<0.001	0.014
Railways	310,943.9	3	51.6	<0.001	0.010	310,973.3	3	52.0	<0.001	0.010
Water bodies	310,944.2	3	51.9	<0.001	0.013	310,973.7	3	52.4	<0.001	0.013
Distance	310,956.4	2	64.1	<0.001	0.012	310,985.5	3	64.2	<0.001	0.026
Motorways	310,956.7	3	64.4	<0.001	0.026	310,985.9	2	64.6	<0.001	0.012
(c) Ten-axes-based factorial correspondence analysis (FCA)										
City border concave	310,842.9		0	0.663	0.389	310,860.3	3	0	0.721	0.386
Built-up areas	310,878.6		35.7	0.211	0.058	310,899.7	3	39.4	0.170	0.057
Forests	310,889.3		46.4	0.095	0.054	310,910.6	3	50.3	0.089	0.052
Arable/green	310,910.9		68.0	<0.001	0.038	310,931.4	3	71.1	0.001	0.037
Water bodies	310,921.5		78.6	0.031	0.172	310,941.0	3	80.7	0.020	0.170
Railways	310,954.5		111.6	<0.001	0.065	310,973.9	3	113.6	<0.001	0.064
Motorways	310,990.1		147.2	<0.001	0.027	311,010.0	3	149.7	<0.001	0.011
Distance	310,991.0		148.1	<0.001	0.012	311,010.1	2	149.8	<0.001	0.011

Notes: Three different genetic distance measures and seven environmental predictors from the German authoritative topographic cartographic information system (ATKIS) were compared (Section 2). The initial model results are presented in Table S14. To check for convergence, each analysis was performed twice for each landscape feature (Run 1 & Run 2). avg. AIC_c, average of the AIC_c values obtained for each model in 1,000 bootstrap iterations; *k*, number of parameters; ΔAIC_c, difference in the avg; AIC_c values between the best supported model (lowest AIC_c) and each subsequent model; avg.weight, average of the AIC_c weights from 1,000 bootstrap iterations; avg.mR^2, average marginal R^2 of 1,000 bootstrap iterations. Predictors are sorted according to increasing avg.AIC_c values

well in model selection accuracy, except in situations of low genetic structure and small sample size. The discrepancies between genetic distance measures reported here are therefore consistent with high dispersal rates.

Shirk et al. (2017) performed Principal Components Analyses (PCA) on multiple contingency tables and found that genetic distances based on multiple-axes PCA maximise model selection accuracy, with other measures performing almost as well in cases of high levels of genetic structure. PCA assumes continuous, normally distributed data (Dytham, 2011), whereas Factorial Correspondence Analysis (FCA) was designed for multistate categorical variables (She, Autemm, Kotulas, Pasteur, & Bonhomme, 1987) and is thus more suitable for the analysis of allele states. Analogous to Shirk et al. (2017), our 10-axes FCA metric led to a better model fit (in terms of marginal R^2) than the other two measures and generated biologically meaningful results. Future research will show whether this is a general feature of FCA and how much this depends on the number of axes included. With a modest strength of the genetic signal, a few large eigenvectors may have insufficient diagnostic power to infer more subtle processes. The geographical distribution of the target species may also matter (Shirk et al., 2017). We considered 10 axes to be a good compromise between accuracy and noise and (almost)

TABLE 4 Results of the multicategorical functional connectivity analysis for the complete data set

Predictors	avg.AIC_c	k	ΔAIC_c	avg.weight	avg.mR^2
City border concave*Built-up areas*Water bodies*Railways	310,624.2	6	0	0.475	0.373
City border concave*Built-up areas*Water bodies	310,624.4	5	0.2	0.371	0.384
City border concave*Built-up areas	310,665.4	4	41.2	0.008	0.397
City border concave*Built-up areas*Arable/Green	310,668.6.	5	44.3	0.138	0.481
City border concave*Built-up areas*Forest	310,675.7	5	51.5	0.008	0.340
City border concave	310,810.5	3	186.3	<0.001	0.384
Built-up areas	310,851.2	3	226.9	<0.001	0.057
Forest	310,862.3	3	238.0	<0.001	0.053
Arable/Green	310,883.1	3	258.9	<0.001	0.037
Water bodies	310,893.7	3	269.5	<0.001	0.170
Railways	310,925.5	3	301.3	<0.001	0.065
Motorways	310,961.1	3	336.9	<0.001	0.027
Distance	310,961.7	2	337.5	<0.001	0.012

Notes: The best-supported multicategorical surfaces combining different environmental predictors were obtained using a stepwise procedure: Individual predictors were added based on model support (corrected Akaike information criterion, AIC_c, values), but only retained if their addition improved support of the multicategorical model ($\Delta AIC_c > 2$). Presented here are the bootstrapping results based on two optimisation runs (summarised in Table S15) that were performed for each (combination of) landscape features. avg. AIC_c, average of the AICc values obtained for each model in 1,000 bootstrap iterations; k, number of parameters; ΔAIC_c, difference in the avg; AIC_c, values between the best supported model (lowest avg.AIC_c) and each subsequent model; avg.weight, average of the AIC_c weights obtained for each model in 1,000 bootstrap iterations; avg.mR^2, average marginal R^2 of 1,000 bootstrap iterations. Predictors are sorted according to inceasing avg.AIC_c values

all single ATKIS predictors had better model support than the distance model alone.

4.3 | Pitfalls in landscape resistance modelling

Our results show that a subtle understanding of gene flow requires the simultaneous consideration of multiple landscape features. However, an issue that emerged as nontrivial was the generation of composite resistance surfaces that include linear features. When considering multiple linear features, model support may depend on the rule for classifying a grid cell where linear features overlap. The comparison of all combinations of environmental predictors using the all _ comb() command was hampered by the way the input grids were created. As we applied a priority rule to linear and a majority rule for shape predictors, they frequently overlapped when generating composite surfaces. This led to the creation of separate categories for each type of overlap and decreased model support. Without a priority rule the linear features would have been interrupted in the input grid. A different option could be to create single-feature input grids by reclassifying a grid containing all features. This poses the problem of how to deal with overlapping linear features and linear features that run in parallel.

As a solution to these dilemmas, we adopted the multicategorical approach where we applied the single-surface optimisation procedure to grid surfaces containing multiple environmental predictors. This allowed us to explicitly test different overlap scenarios. In combination with the stepwise approach of creating multicategorical surfaces, this required fewer optimisation runs than the comparison of all possible combinations using all _ comb(). This might be an important consideration when having a large(r) number of predictors. A drawback of the multicategorical approach was that model support and optimised resistance values were sensitive to starting values of the input surface. The method(s) for simultaneously considering multiple landscapes therefore need(s) to be chosen carefully.

Finally, the layers in this study were very general. For example, we assumed that every stretch of motorway as well as every Urban Atlas category had a consistent effect on gene flow over space and time. We are well aware that this approach reflects the actual structure of the habitat only to some extent, especially in the urban area: The already highly heterogeneous structure of a rapid growing metropolis like Berlin is subject to permanent fluctuation. These processes cannot be reflected in the spatial data set and sometimes the same environmental predictors may even have opposite effects on gene flow. A highway in Berlin, for example, may be a strong barrier if it is fenced-in and rarely interrupted by bridges or underpasses. In contrast, the same motorway may serve as a corridor on other stretches, if it is combined with long, continuous green strips connecting highly fragmented built-up areas. Consequently, those layers can only serve as an approximation of the functional connectivity of the real landscape. Nevertheless, this may still yield valuable insights into dispersal processes.

4.4 | The urban island

Our results provided general support for a genetic differentiation between urban and rural areas, i.e. the urban island hypothesis. The observed genetic structure was relatively weak, indicating that some individuals from the surrounding areas do disperse into Berlin. With abundant high-quality food and a lack of hunting pressure, the city is possibly a better-quality habitat for foxes, despite an increased mortality. Urban foxes could therefore be expected to stay within the city and individuals from the surrounding areas to disperse into the urban area. However, there was no support for a constant influx of foxes from the countryside and the (genetic) exchange between the urban agglomeration and the rural countryside was sufficiently reduced to maintain genetic structure. In line with this, a radio-tracking study of foxes in Zurich showed limited movement across an the urban-rural boundary (Gloor, 2002). Colonising urban areas may thus require behavioural shifts such as an improved tolerance of the presence of humans (Gloor et al., 2001). Such behavioural changes have often been interpreted as resulting from phenotypic plasticity, allowing habituation to humans (Bateman & Fleming, 2012; Kauhala, Talvitie, & Vuorisalo, 2016; Vuorisalo, Talvitie, Kauhala, Bläuer, & Lahtinen, 2014). However, work on urban birds suggested that avoidance of humans may have a genetic basis and urban colonisation may result from selection for fearless individuals (Carrete et al., 2016; Carrete & Tella, 2009; Møller et al., 2015). The presence of a genetically distinct urban population may thus result from a founder effect followed by limited urban-rural exchange due to differences in avoidance behaviour (see also below).

Given the political history of Berlin, there remains another explanation for the presence of an urban cluster: Between 1961 and 1989 the “Berlin Wall” (partially following the river Spree) separated West Berlin from eastern Berlin and the surrounding federal state of Brandenburg. While a founder effect may have created an initial reduction in genetic diversity among urban foxes, impermeable border fortifications could have limited gene flow and thus artificially maintained genetic differentiation between urban and rural foxes. However, genetic exchange between urban and rural foxes must also have remained sufficiently low in the ensuing 30 years to maintain genetic structure (with generation time being 2–3 years, DeCandia et al., 2019). Based on F_{ST} values ($F_{ST} \geq 0.027$), Wandeler et al. (2003) detected genetic differentiation between urban and rural foxes for the then recently (15 years) established fox population within Zurich. However, assignment tests provided evidence for ongoing urban-rural gene flow. A recent re-analysis of the same data set identified only one evolutionary cluster (DeCandia et al., 2019). Further research in other metropolitan areas might help to clarify whether the urban island is a general phenomenon or a specificity of Berlin.

4.5 | Gene flow within the cityscape

Gene flow in Berlin foxes was hampered by physical barriers. The landscape resistance models identified major water bodies as the most significant predictor resisting gene flow in the urban area. Contrary to our predictions, foxes did not freely move through the urban landscape. The best-supported multicategorical model(s) inferred highly urbanised areas (sealing levels >80%) to represent an important impediment to gene flow. On the other hand, urban fox dispersal did not depend on corridors of natural vegetation as it was described for other species (Goldingay et al., 2013; Munshi-South, 2012) either. While suburban areas with low degrees of imperviousness were inferred to be more permeable for dispersers than densely built-up areas, our results suggest that railways and motorways served as the main dispersal corridors. This last result is in line with results from radio-tracking studies in Edinburgh where railway lines were the main conduit for long-distance dispersal of male foxes (Kolb 1984).

Railway lines and motorways are highly artificial structures. On the circular railway around the city centre, trains pass continuously day and night. Similarly, the multilane motorways connecting the districts of Berlin are extremely busy with high-speed traffic. While railway-tracks are usually embedded within vegetated verges, motorways are not, and generally, dispersal along such transport infrastructure carries a high mortality risk (200–250 road-killed foxes are found in Berlin each year: Börner, 2014). Yet, what both landscape elements have in common (besides their linearity), is the absence of human activity, in terms of pedestrians and cyclists. The green spaces of Berlin, in contrast, are usually crowded. Although the actual mortality risk in green spaces and sparse built-up areas is low, they show less conductance to gene flow than motorways and railways (Figure 3), despite the latter's inherent mortality risk. Consequently, foxes may use artificial structures as corridors but avoid areas of human activity (see also Table 5). Adkins and Stott (1998) reported that city foxes stayed shy and preferably used sites when human activity was low. The authors concluded that foxes do not avoid human constructions—but humans themselves. Beyond physical barriers, human activity may thus represent a significant impediment to dispersal in urban foxes.

4.6 | The landscape of fear

Over centuries, foxes have been intensively hunted by humans - and still are. Although no hunting is conducted within the city, foxes should thus maintain a certain level of “background fear” (see

TABLE 5 Detected landscape resistance versus expected resistance effect of environmental predictors under the assumption of disturbance due to artificiality of the predictor or disturbance due to associated human activity and detected resistance pattern

	Expected effect of the environmental predictor		
Predictor	Disturbance due to manmade structures (signs of human neighbourhood)	Disturbance due to human presence (human activity)	Detected resistance/ conductance
Motorways	High resistance	High conductance	High conductance
Railways	Medium resistance	High conductance	High conductance
Green spaces	High conductance	Medium resistance	Low conductance

Laundré, Hernández, & Ripple, 2010). The concept of a "landscape of fear" (Laundré, Hernández, & Altendorf, 2001; Laundré et al., 2010) is frequently applied to foraging behaviour and predator-prey relationships, but the authors promote its consideration for various life history traits. It describes how fear (or predator-induced stress) affects how animals use landscapes. It is not the actual predation risk but the anticipation of risks that limits movement in a landscape of fear (Laundré et al., 2010; Lima, 1998). In the context of our study, this could indicate that human activity drives urban foxes into costly trade-offs as they primarily disperse along structures with little human activity (hence low perceived risk) but high inherent mortality risks. This result conflicts with a model of fearless individuals entering and roaming through the city. Rather, behavioural plasticity may have allowed some foxes to enter the city and facilitate habituation to human presence to some extent, modifying but not obliterating their landscape of fear.

Movement constraints imposed by human activity could be even more relevant for rural foxes that are less accustomed to human presence (see also Stillfried, Gras, et al., 2017). Our results show that rural foxes, unlike their city relatives, do not use artificial structures as dispersal corridors and that dispersal was limited by the city border (Figure 3). It may thus not be the rural foxes' physical capacity to move but the fear to do so that hinders rural foxes from entering the urban island and prevents admixture.

No matter how the genetic differentiation arose, the urban island could persist due to additional behavioural movement limitations. Human presence may thus be the key driver of red fox dispersal behaviour and impact both the separation into rural and urban clusters as well as the dispersal processes within the urban area.

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AUTHOR CONTRIBUTIONS

A.C.F., K.B., and S.E.K. contributed to the design of this research. C.S., K.B., M.H., and U.W. collected the samples. AS and TH performed the laboratory work. C.St., and J.B. contributed to the generation of the resistance surfaces. A.C.F., and S.E.K. conducted data analysis. S.E.K. wrote the manuscript. A.C.F., J.B., M.B., H.H., S.K.S. contributed with substantial revisions to the manuscript.

DATA AVAILABILITY STATEMENT

Microsatellite genotypes, Geographic coordinates, all ascii grid files, and R code for the running of RESISTANCEGA are available on Dryad (https://doi.org/10.5061/dryad.dv41ns1ts).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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MOLECULAR ECOLOGY

Supplemental Information for:

Beyond the landscape: resistance modelling infers physical and behavioural gene flow barriers to a mobile carnivore across a metropolitan area.

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Table of Contents:

Appendix S1: Methods used for genotyping 16 microsatellite loci

DNA was extracted using an ammonium acetate-based salting-out procedure (S. A. Miller, Dykes, & Polesky, 1988). DNA extracts were quantified using a Drop-Sense 16 spectrophotometer (Trinean, Gentbrugge, Belgium). We used 16 microsatellite loci that were amplified in three Polymerase Chain Reactions (PCR). Multiplex 1 contained loci DGN14, REN69B24, V374*, V402, Vv-C01.424 and VVM189. Multiplex 2 contained loci FH2541*, Vv-CPH11, Vv-INU055 and VVM124. Multiplex 3 contained loci DGN3*, REN161A12*, REN162B09, V502*, Vv-C08.618 and Vv-CPH2. The 5'-ends of the reverse primers of the loci marked with an asterisk were labelled with the 'pigtail' sequence GTTTCTT to limit noise from variable adenylation during PCR (Brownstein, Carpten, & Smith, 1996). Each PCR contained 1 x QIAGEN Multiplex Master Mix and 0.2 µM of each primer (except VVM189 at 0.4 µM). PCRs started with 5 min denaturation at 95 °C, followed by cycles of denaturation at 95 °C for 30 s, annealing for 90 s and extension at 72 °C for 90 s. For all three multiplexes, the initial annealing temperature of 64 °C was reduced by one degree every cycle for six cycles. This was followed by 29 cycles of annealing at 58 °C. The final incubation was at 68 °C for 10 min. The PCRs were performed in a Mastercycler nexus (Eppendorf, Hamburg, Germany). PCR products were genotyped using a capillary sequencer (ABI 3730XL, Applied Biosystems). Allele sizes were determined using GENEMAPPER version 4.0 (Applied Biosystems). The genetic profiles of all samples consisted of at least 11 loci.

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Appendix S2: Generation of ten subsampled data sets consisting of 24 spatially clustered individuals.

To avoid deviations from Hardy-Weinberg equilibrium (HWE) resulting from Wahlund effects and isolation-by-distance, we tested the 16 microsatellite loci in ten subsampled data sets each consisting of 24 spatially clustered individuals. A data set was obtained by randomly selecting an individual from the complete dataset of 387 individuals and including its 23 nearest geographic neighbours. We aimed to avoid that any two of the ten subsamples shared more than two individuals between them, and we therefore repeatedly generated 15 datasets until ten of these 15 datasets fulfilled this criterion. We used the INTERSECT() function in program R v3.5.2 (Ihaka & Gentleman 1996) to estimate the number of individuals shared between each combination of datasets. In the final selection, only one pair of datasets shared (two) individuals (Fig. A).

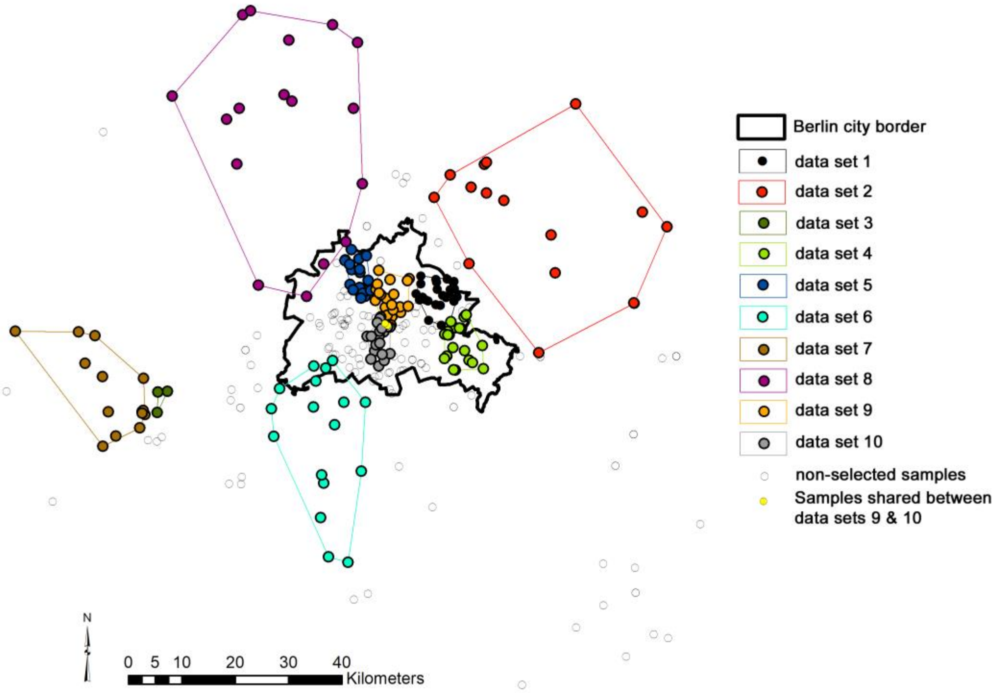

Fig. A: Location of the ten subsamples of 24 individuals (data set 1 to 10) used to test the 16 microsatellite loci for deviations from Hardy-Weinberg and linkage equilibria. One point can represent more than one individual. Two geographic outlier individuals are omitted from the plot.

Reference:

Ihaka, R., & Gentleman, R. (1996). A language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, *5*, 299-314.

Appendix S3: Running modalities of the Bayesian clustering analyses.

In order to estimate the number of genetic subpopulations (*K*) using STRUCTURE, ten independent runs of *K*=1-10 were conducted with 10^6 Markov chain Monte Carlo (MCMC) iterations after a 10^5-iteration burn-in length, using the admixture and correlated-allele-frequency models. ALPHA, the Dirichlet parameter for the degree of admixture, was allowed to vary between subpopulations and set be non-uniform (priors: α=0.05, β=0.001). The most probable number of subpopulations was chosen based on the ten log-likelihood values inferred for each *K* and their convergence. The assignment of individuals to the detected clusters was calculated by averaging each individual's proportion of membership over the ten runs. To determine the number of genetic clusters, the GENELAND algorithm was run 10 times, with *K*=1-10 and 10^6 MCMC iterations with a thinning of 1000, maximum rate of the Poisson process equal to sample size, maximum number of nuclei in the Poisson-Voronoi tessellation equal to three times the sample size and uncertainty attached to the spatial coordinates fixed at 250m. The Dirichlet model served as a prior for all allele frequencies. Next, the algorithm was run an additional 100 times with a fixed value for *K*, according to the inferred number of clusters, conducting 10^6 MCMC iterations with thinning of 1000 and all further parameters as described above.

Appendix S4: Running modalities of the Estimated Effective Migration Surfaces (EEMS) method

The Estimated Effective Migration Surfaces method uses a stepping stone model to evaluate whether migration rates between neighbouring demes are higher than expected and interpolates a surface illustrating effective migration between and effective diversity within each deme. We ran the method simulating 1000 demes, after first adjusting the parameters until reaching a recommended proportion of acceptance of 20-30%: mEffctProposalS2 = 5, mSeedsProposalS2 = 0.18, mrateMuProposalS2 = 0.20, qEffctProposalS2 = 0.032, qSeedsProposalS2 = 0.14. We performed ten independent runs with 107 iterations, sampling every 9999th iteration after a burn-in of 2x106 iterations.

Table S1: Land use categories employed in the 2012 Copernicus Urban Atlas. In the Urban atlas, cities are mapped at a scale of 1:10.000, with the smallest identifiable surface being 0.25 ha in urbanised areas and 1 ha in non-urbanised areas. The minimum accuracy of the data is 85 % for artificial surfaces and 80 % for the other classes.

1. Artificial surfaces		
11	Urban Fabric	
	11100	Continuous urban fabric (S.L.: >80%)
	11210	Discontinuous dense urban fabric (S.L. 50%-80%)
	11220	Discontinuous medium density urban fabric (S.L. 30%-50%)
	11230	Discontinuous low density urban fabric (S.L. 10%-30%)
	11240	Discontinuous very low density urban Fabric (S.L. <10%)
	11300	Isolated structures
12	Industrial, commercial, public, military and private units	
	12100	Industrial, commercial, public, military and private units
	12210	Fast transit roads and associated lands
	12220	Other roads and associated lands
	12230	Railways and associated lands
	12300	Port areas
	12400	Airports
	Mine, dump and construction sites	
	13100	Mineral extraction and dump sites
	13300	Construction sites
	13400	Land without current use
	Artificial non-agricultural vegetated areas	
	14100	Green urban areas
	14200	Sports and Leisure facilities
2. Agricultural and Semi-natural		
	21000	Arable land (annual crops)
	22000	Permanent crops
	23000	Pastures
	24000	Complex and mixed cultivation patterns
	25000	Orchards
3. Forest (natural and plantation)		
	31000	Forests
	32000	Herbaceous vegetation associations
	33000	Open spaces with little or no vegetation
4. Wetlands	40000	Wetlands
5 Water	50000	Water

Table S2: Significance values of the GENEPOP exact test for Hardy-Weinberg deviations in 10 datasets of 24 spatially clustered individuals. The data sets were obtained by randomly selecting an individual from the complete dataset of 387 individuals and including its 23 nearest geographic neighbours (see also Appendix 2). Values that are underlined were significant before the multiple-test correction, while values in bold were still significant after this correction. DSet 1 to DSet 10: Subsampled data sets 1 to 10 (corresponding to dataset 1 to 10 shown in Fig. A of Appendix 2).

microsatellite	**Subsampled data sets**									
locus	**DSet 1**	**DSet 2**	**DSet 3**	**DSet 4**	**DSet 5**	**DSet 6**	**DSet 7**	**DSet 8**	**DSet 9**	**DSet 10**
DGN14	**<0.001**	0.400	**0.005**	0.039	0.802	0.252	0.707	0.089	0.863	0.670
DGN3	0.234	0.015	0.015	0.008	0.132	0.057	0.049	0.049	**<0.001**	0.134
FH2541	0.041	0.865	0.523	0.666	0.452	0.321	0.407	0.267	0.999	0.769
REN161A12	0.162	0.807	0.949	0.190	0.234	0.471	0.930	0.231	0.835	0.642
REN162B09	0.291	0.572	0.695	0.765	0.177	1.000	0.033	0.228	1.000	0.110
REN69B24	0.653	0.575	**0.005**	0.928	0.176	0.184	0.085	0.913	0.053	0.083
V374	0.232	0.856	0.279	0.523	0.135	0.164	0.167	0.768	0.434	0.265
V402	0.243	0.822	0.544	0.303	0.010	0.456	0.896	0.470	0.152	0.519
V502	0.052	0.049	**0.001**	0.011	**<0.001**	**<0.001**	**0.001**	**0.002**	0.010	1.000
VV-C01.424	0.247	0.628	0.665	0.812	0.107	0.356	0.697	0.042	0.742	0.650
VV-C08.618	0.471	0.504	0.237	0.639	0.121	0.158	0.895	0.033	0.490	0.189
VV-CPH11	0.118	1.000	0.453	0.663	0.583	0.536	0.399	0.315	0.053	1.000
VV-CPH2	0.400	0.659	0.377	0.264	0.881	1.000	0.701	0.689	0.222	0.424
VVINU055	0.349	0.455	0.112	0.448	0.830	0.860	0.108	0.023	0.786	0.612
VVM124	0.918	0.417	0.415	0.548	0.028	0.797	0.041	0.742	0.772	0.053
VVM189	0.021	0.007	0.775	0.044	0.601	0.596	0.009	0.009	**0.001**	**0.003**

Table S3: Results of the GENEPOP exact test for Linkage Disequilibrium (LD). Significant pairs and corrected *p*-values among the 120 primer pairs, in 10 subsampled data sets each consisting of 24 spatially clustered individuals (see Appendix 2).

Simulated population	**locus 1**	**locus 2**	**p-value**
run III out of X	DGN14	REN161A1	<0.001
run IX out of X	V402	VVM189	<0.001
run IX out of X	DGN*	REN162B09	<0.001
run X out of X	FH2541	REN161A12	<0.001

Table S4: Summary statistics for clustering results. Estimates of genetic diversity in the two inferred genetic clusters from GENELAND and STRUCTURE (see also Figs. 1c & 1d). ***N***: number of individuals, ***He***: expected heterozygosity, ***Ho***: observed heterozygosity, ***A***: average number of alleles across loci. The colour information in column one is indicative of the identity of the cluster in Figs. 1c & 1d.

Software	**Cluster**	***N***	**H_e**	**H_o**	***A***
GENELAND	Berlin (orange)	203	0.78	0.74	10.7
	Brandenburg (blue)	171	0.77	0.71	11.7
STRUCTURE	Central Berlin (orange)	118	0.73	0.70	9.3
	Other (blue)	256	0.78	0.73	11.9

Table S5: Initial pre-bootstrapping results of the single-predictor ResistanceGA analysis for the city of Berlin. Three different genetic distance measures and five environmental predictors from the German authoritative topographic cartographic information system (ATKIS) were compared (see Material and Methods). To check for convergence, each opimisation was performed twice for each landscape feature (Run 1 & Run 2). Predictors are sorted according to increasing corrected Akaike information criterion (AIC$_c$) values. ***k*** = number of parameters, **ΔAIC$_c$** = difference in the AIC$_c$ values between the best supported model (lowest AIC$_c$) and each subsequent model. **m*R*^{2}** = marginal R^2, **Resistance** = resistance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation).

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**	**Resistance**		**Predictor**	**AIC$_c$**	**k**	**ΔAIC$_c$**	**m*R*^{2}**	**Resistance**	
					Matrix	**Predictor**						**Matrix**	**Predictor**
Run 1							Run 2						
a) Nei's genetic distance (Nei)													
Motorways	-38935.2	3	0	0.036	500	1	Motorways	-38935.8	3	0	0.036	589	1
Railways	-38911.2	3	24.0	0.058	33	1	Railways	-38911.2	3	24.6	0.058	33	1
Distance	-38906.1	2	29.1	0.018			Distance	-38906.1	2	29.7	0.018		
Water bodies	-38905.7	3	29.5	0.022	1	2	Water bodies	-38905.7	3	30.1	0.022	1	2
Built-up areas	-38904.5	3	30.7	0.017	1	1	Built-up areas	-38904.5	3	31.3	0.017	1	1
All vegetation	-38904.0	3	31.2	0.017	1	1	All vegetation	-38904.1	3	31.7	0.017	1	1
Null	-38647.3	1	287.9	0			Null	-38647.3	1	288.5	0		
b) Proportion of shared alleles (D_{PS})													
Motorways	-38897.9	3	0	0.024	31	1	Motorways	-38914.7	3	0	0.033	323	1
Railways	-38895.6	3	2.3	0.055	31	1	Railways	-38895.6	3	19.1	0.055	31	1
Water bodies	-38891.5	3	6.4	0.052	82	1	Distance	-38889.8	2	24.9	0.017		
Distance	-38889.8	2	8.1	0.017			Water bodies	-38888.9	3	25.8	0.020	1	2
Built-up areas	-38888.4	3	9.5	0.017	1	1	Built-up areas	-38888.4	3	26.3	0.017	1	1
All vegetation	-38887.9	3	10.0	0.017	1	1	All vegetation	-38887.9	3	26.8	0.017	1	1
Null	-38642.6	1	255.3	0			Null	-38642.6	1	272.1	0		
c) 10-axes based Factorial Correspondence Analysis (FCA)													
Water bodies	232528.8	3	0	0.303	1	44	Water bodies	232528.8	3	0	0.303	1	44
Railways	232577.5	3	48.7	0.055	99	1	Railways	232577.5	3	48.7	0.055	99	1
Motorways	232593.0	3	64.2	0.014	500	1	Motorways	232592.9	3	64.1	0.014	500	1
All vegetation	232625.9	3	97.1	0.029	9	1	All vegetation	232626.0	3	97.2	0.026	8	1
Built-up areas	232632.2	3	103.4	0.028	1	6	Built-up areas	232632.2	3	103.4	0.028	1	7
Distance	232643.5	2	114.7	0.006			Distance	232643.5	2	114.7	0.006		
Null	233094.8	1	566.0	0			Null	233094.8	1	566.0	0		

Table S6: Initial pre-bootstrapping results from the optimisation of all possible combination of the five single ATKIS environmental predictors (ALL_COMB() function). To check for convergence, each optimisation was performed twice for each (ccombination of) landscape feature (Run 1 & Run 2). **AIC$_c$** = corrected Akaike information criterion, ***k*** = number of parameters, **ΔAIC$_c$** = difference in the AIC$_c$ values between the best supported model (lowest AIC$_c$) and each subsequent model. **m*R*^{2}** = marginal R^2.

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**	**Predictor**	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**
a) Run 1					b) Run 2				
Built-up areas*Water bodies	232522.3	5	0	0.296	Built-up areas*Water bodies	232522.5	5	0	0.296
Vegetation*Water bodies	232524.3	5	2.0	0.301	Vegetation*Water bodies	232524.2	5	1.7	0.289
Railways*Water bodies	232526.3	5	4.0	0.262	Railways*Water bodies	232526.4	5	3.9	0.073
Built-up areas*Railways*Water bodies	232528.4	7	6.1	0.306	Built-up areas*Motorways*Water bodies	232527.9	7	5.4	0.275
Built-up areas*Motorways*Water bodies	232528.7	7	6.4	0.289	Built-up areas*Railways*Water bodies	232527.9	7	5.4	0.282
Water bodies	232528.8	3	6.5	0.303	Water bodies	232528.8	3	6.3	0.303
Built-up areas*Vegetation*Water bodies	232529.4	7	7.1	0.280	Railways*Vegetation*Water bodies	232529.3	7	6.8	0.293
Motorways*Vegetation*Water bodies	232529.5	7	7.2	0.293	Built-up areas*Vegetation*Water bodies	232529.4	7	6.9	0.288
Motorways*Water bodies	232529.5	5	7.2	0.295	Motorways*Water bodies	232529.5	5	7.0	0.294
Railways*Vegetation*Water bodies	232529.7	7	7.4	0.298	Motorways*Vegetation*Water bodies	232529.8	7	7.3	0.280
Motorways*Railways*Water bodies	232534.0	7	11.7	0.291	Motorways*Railways*Water bodies	232534.2	7	11.7	0.290
Built-up areas*Motorways*Vegetation*Water bodies	232534.5	9	12.2	0.285	Built-up areas*Motorways*Railways*Water bodies	232535.3	9	12.8	0.280
Built-up areas*Motorways*Railways*Water bodies	232535.4	9	13.1	0.302	Built-up areas*Railways*Vegetation*Water bodies	232535.7	9	13.2	0.298
Motorways*Railways*Vegetation*Water bodies	232535.5	9	13.2	0.295	Built-up areas*Motorways*Vegetation*Water bodies	232536.1	9	13.6	0.276
Built-up areas*Railways*Vegetation*Water bodies	232536.7	9	14.4	0.300	Motorways*Railways*Vegetation*Water bodies	232536.5	9	14.0	0.291
Built-up areas*Motorways*Railways*Vegetation* Water bodies	232541.3	11	19.0	0.282	Built-up areas*Motorways*Railways*Vegetation* Water bodies	232541.8	11	19.3	0.284
Railways*Vegetation	232574.3	5	52.0	0.052	Railways*Vegetation	232574.3	5	51.8	0.052
Railways	232577.5	3	55.2	0.055	Railways	232577.5	3	55.0	0.055
Built-up areas*Railways	232579.1	5	56.8	0.051	Built-up areas*Railways	232577.8	5	55.3	0.056
Motorways*Railways	232579.4	5	57.1	0.057	Motorways*Railways	232579.5	5	57.0	0.058
Motorways*Railways*Vegetation	232581.1	7	58.8	0.053	Motorways*Railways*Vegetation	232580.8	7	58.3	0.051
Built-up areas*Motorways*Railways	232581.9	7	59.6	0.055	Built-up areas*Railways*Vegetation	232581.8	7	59.3	0.050
Built-up areas*Railways*Vegetation	232582.6	7	60.3	0.049	Built-up areas*Motorways*Railways	232581.9	7	59.4	0.053
Built-up areas*Motorways*Railways*Vegetation	232588.6	9	66.3	0.054	Built-up areas*Motorways*Railways*Vegetation	232587.5	9	65.0	0.051
Motorways	232593.0	3	70.7	0.014	Motorways*	232593.0	3	70.5	0.014
Built-up areas*Motorways	232603.3	5	81.0	0.013	Motorways*Vegetation	232603.3	5	80.8	0.013
Motorways*Vegetation	232603.3	5	81.0	0.013	Built-up areas*Motorways	232603.3	5	80.8	0.013
Built-up areas*Motorways*Vegetation	232612.4	7	90.1	0.012	Built-up areas*Motorways*Vegetation	232612.4	7	89.9	0.012
Vegetation	232626.0	3	103.7	0.026	Vegetation	232625.9	3	103.4	0.029
Built-up areas*Vegetation	232629.3	5	107.0	0.029	Built-up areas*Vegetation	232629.8	5	107.3	0.026
Built-up areas	232632.2	3	109.9	0.028	Built-up areas	232632.2	3	109.7	0.028
Distance	232643.5	2	121.2	0.006	Distance	232643.5	2	121.0	0.006
Null	233094.8	1	572.5	0.000	Null	233094.8	1	572.3	0.000

Table S7: Boostrap results from the optimisation of all possible combination of the five single ATKIS environmental predictors (ALL_COMB() function). In the initial analysis (summarised in Table S6), the optimisation procedure was repeated twice for each landscape feature. For each combination of landscape feature, we only included the distance matrix from the run with the lowest corrected Akaike information criterion (AIC$_c$) in the bootstrap analysis. **avg. AIC$_c$** = average of the AICc values obtained for each model in 1000 bootstrap iterations. ***k*** = number of parameters, **ΔAIC$_c$** = difference in the avg. AIC$_c$ values between the best supported model (lowest AIC$_c$) and each subsequent model. **avg.weight** = average of the AIC$_c$ weights obtained for each model in 1000 bootstrap iterations. **avg.m*R*^{2}** = average marginal R^2 of 1000 bootstrap iterations. Predictors are sorted according to increasing averaged AIC$_c$ values.

Predictor	**avg. AIC$_c$**	***k***	**ΔAIC$_c$**	**avg.weight**	**m*R*^{2}**
Railways*Water bodies	130723.9	5	0.0	0.585	0.074
Built-up areas*Water bodies	130732.8	5	8.9	0.116	0.283
Built-up areas*Vegetation*Water bodies	130733.9	7	9.9	0.026	0.268
Built-up areas*Motorways*Water bodies	130733.9	7	10.0	0.028	0.277
Motorways*Vegetation*Water bodies	130734.4	7	10.5	0.024	0.268
Vegetation*Water bodies	130734.4	5	10.5	0.053	0.288
Built-up areas*Railways*Water bodies	130734.6	7	10.7	0.033	0.293
Railways*Vegetation*Water bodies	130735.2	7	11.3	0.021	0.285
Built-up areas*Motorways*Vegetation*Water bodies	130735.5	9	11.6	0.011	0.265
Motorways*Water bodies	130736.4	5	12.4	0.024	0.282
Built-up areas*Motorways*Railways*Water bodies	130736.5	9	12.6	0.011	0.289
Motorways*Railways*Vegetation*Water bodies	130736.6	9	12.7	0.009	0.279
Motorways*Railways*Water bodies	130736.6	7	12.7	0.017	0.278
Built-up areas*Railways*Vegetation*Water bodies	130737.1	9	13.2	0.008	0.286
Built-up areas*Motorways*Railways*Vegetation*Water bodies	130737.3	11	13.4	0.006	0.272
Water bodies	130738.7	3	14.7	0.009	0.290
Railways*Vegetation	130750.2	5	26.3	0.001	0.053
Motorways*Railways*Vegetation	130752.1	7	28.1	0.001	0.054
Built-up areas*Motorways*Railways	130752.5	7	28.6	0.001	0.055
Built-up areas*Railways	130752.8	5	28.9	0.001	0.053
Built-up areas*Railways*Vegetation	130752.8	7	28.9	0.000	0.050
Motorways*Railways	130753.2	5	29.3	0.001	0.059
Railways	130754.0	3	30.1	<0.001	0.057
Built-up areas*Motorways*Railways*Vegetation	130754.2	9	30.3	<0.001	0.055
Motorways	130763.0	3	39.1	0.010	0.014
Motorways*Vegetation	130766.7	5	42.8	0.002	0.013
Built-up areas*Motorways	130766.7	5	42.8	0.002	0.013
Built-up areas*Motorways*Vegetation	130769.9	7	45.9	<0.001	0.012
Vegetation	130783.2	3	59.3	<0.001	0.027
Built-up areas*Vegetation	130783.3	5	59.4	<0.001	0.027
Built-up areas	130786.0	3	62.1	<0.001	0.029
Distance	130792.8	2	68.9	<0.001	0.007

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Table S8: Results of single-surface optimisation (SS_OPTIM()) procedures with two linear environmental predictors (see Materials and Methods), modelling different overlap scenarios. Results are for the Berlin-only dataset, using the FCA-based genetic distance measure. (i), (ii): Initial pre-bootstrapping results. Optimisation was performed twice for each combination of landscape features; (iii) Bootstrapping results. For each combination of landscape feature, we only included the distance matrix from the run (marked with ‘^{x}’) with the lowest corrected Akaike information criterion (**AIC$_c$**) in the bootstrap analysis. “>” indicates which predictor took priority, meaning that every grid cell containing a point of overlap with other linear predictors, the cell was codified as belonging to the predictor with priority. ‘*Bridges*’ predictor: A cell with two overlapping linear predictors was classified as a distinct predictor. Predictors are sorted according to increasing AIC$_c$ values. **avg. AICc** = average of the AICc values obtained for each model in 1000 bootstrap iterations. **ΔAICc** = difference in the (avg.) AICc values between the best supported model and each subsequent model. ***k*** = number of parameters, **m*R*^{2}** = marginal *R*^{2}, **avg.m*R*^{2}** = average marginal *R*^{2} of 1000 bootstrap iterations. **avg.weight** = average of the AICc weights obtained for each model in 1000 bootstrap iterations. **Resistance** = resistance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation).

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**	**Resistance**
(i) first analysis					
Water bodies>Railways	232514.9	4	0	0.085	Rail: 1, Matrix: 111, Water: 500
Bridges	232516.5	5	1.6	0.211	Rail: 1, Matrix: 28, Overlap: 405, Water: 494
Water bodies	232528.8	3	13.9	0.303	Matrix: 1, Water: 44
^{x}Railways>Water bodies	232561.8	4	46.9	0.121	Rail: 1, Matrix: 63, Water: 416
Distance	232643.5	2	128.6	0.006	
Null	233094.8	1	579.9	0	
(ii) repeat analysis					
^{x}Water bodies> Railways	232514.9	4	0	0.088	Rail: 1, Matrix: 105, Water: 500
^{x}Bridges	232516.1	5	1.2	0.226	Rail: 1, Matrix: 25, Overlap: 249, Water: 499
^{x}Water bodies	232528.8	3	13.9	0.303	Matrix: 1, Water: 44
Railways>Water bodies	232561.8	4	46.9	0.120	Rail: 1, Matrix: 105, Water: 416
Distance	232643.5	2	128.6	0.006	
Null	233094.8	1	579.9	0	

Predictor	**avg. AIC$_c$**	***k***	**ΔAIC$_c$**	**avg.weight**	**avg.m*R*^{2}**
(iii) bootstrapping					
Water bodies>Railways	130723.1	4	0	0.705	0.089
Bridges	130728.6	5	5.5	0.268	0.219
Water bodies	130742.4	3	19.3	0.025	0.290
Railways>Water bodies	130750.0	4	26.9	0.002	0.121
Distance	130795.6	2	72.5	<0.001	0.007

Table S9: Results of single-surface optimisation (SS_OPTIM()) procedures with three linear environmental predictors (see Materials and Methods), modelling different overlap scenarios. Results are for the Berlin-only dataset, using the FCA-based genetic distance measure. (i), (ii): Initial pre-bootstrapping results. Each optimisation was performed twice for each combination of landscape features; (iii) Bootstrapping results. For each combination of landscape features, we only included the distance matrix from the run (marked with an ‘^{x}’) with the lowest corrected Akaike information criterion (**AIC$_c$**) in the bootstrap analysis. “>” indicates which predictor took priority, meaning that every grid cell containing a point of overlap with other linear predictors, the cell was codified as belonging to the predictor with priority. ‘*Bridges*’ predictor: A cell with two overlapping linear predictors was classified as a distinct predictor. **avg. AICc** = average of the AICc values obtained for each model in 1000 bootstrap iterations. **ΔAICc** = difference in the (avg.) AICc values between the best supported model and each subsequent model. ***k*** = number of parameters, **m*R*^{2}** = marginal R^2, **avg.m*R*^{2}** = average marginal R^2 of 1000 bootstrap iterations. **avg.weight** = average of the AICc weights obtained for each model in 1000 bootstrap iterations. **Resistance** = resistance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation). Predictors are sorted according to increasing AIC$_c$ values.

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**	Resistance
(i) first analysis					
Water bodies>Motorways>Railways	232498.4	5	0	0.139	Motorways: 1, Rail: 23, Matrix: 1278, Water: 9376
^{x}Motorways>Water bodies>Railways	232500.7	5	2.3	0.135	Motorways: 1, Rail: 2, Matrix: 192, Water: 1077
^{x}Water bodies>Railways>Motorways	232501.5	5	3.1	0.111	Motorways: 1, Rail: 4, Matrix: 376, Water: 2011
Bridges	232514.6	6	16.2	0.100	Motorways: 1, Rail: 1, Matrix: 96, Bridges: 453, Water: 526
Water bodies>Railways	232514.9	4	16.5	0.085	Rail: 1, Matrix: 111, Water: 500
^{x}Railways>Water bodies>Motorways	232541.9	5	43.5	0.158	Motorways: 1, Rail: 6, Matrix: 430, Water: 2737
^{x}Motorways>Railways >Water bodies	232544.9	5	46.5	0.133	Motorways: 1, Rail: 13, Matrix: 298, Water: 2349
Railways>Motorways >Water bodies	232559.1	5	60.7	0.090	Rail: 1, Motorways: 2, Matrix: 54, Water: 271
Distance	232643.5	2	145.1	0.006	
Null	233094.8	1	596.4	0.000	
(ii) repeat analysis					
^{x}Water bodies>Motorways>Railways	232495.7	5	0	0.197	Motorways: 1, Rail: 101, Matrix: 4000, Water: 46145
Motorways>Water bodies>Railways	232500.9	5	5.2	0.150	Motorways: 1, Rail: 3, Matrix: 250, Water: 1605
Water bodies>Railways>Motorways	232504.3	5	8.6	0.093	Motorways: 1, Rail: 8, Matrix: 941, Water: 3398
^{x}Bridges	232512.8	6	17.1	0.102	Motorways: 1, Rail: 2, Matrix: 155, Bridges: 665, Water: 848
^{x}Water bodies>Railways	232514.9	4	19.2	0.088	Rail: 1, Matrix: 105, Water: 500
Motorways>Railways >Water bodies	232547.1	5	51.4	0.127	Motorways: 1, Rail: 5, Matrix: 322, Water: 1491
^{x}Railways>Motorways >Water bodies	232547.3	5	51.6	0.156	Motorways: 1, Rail: 3, Matrix: 171, Water: 1176
Railways>Water bodies>Motorways	232548.6	5	52.9	0.093	Motorways: 1, Rail: 2, Matrix: 141, Water: 554
Distance	232643.5	2	147.8	0.006	
Null	233094.8	1	599.1	0.000	

Predictor	**avg. AIC$_c$**	***k***	**ΔAIC$_c$**	**avg.weight**	**avg.m*R*^{2}**
(iii) bootstrapping					
Water bodies>Railways>Motorways	130741.4	5	0	0.205	0.112
Motorways>Water bodies>Railways	130741.5	5	0.1	0.366	0.136
Water bodies>Motorways>Railways	130741.7	5	0.3	0.365	0.195
Bridges	130746.8	6	5.4	0.032	0.104
Water bodies>Railways	130749.6	4	8.2	0.025	0.090
Railways>Water bodies>Motorways	130765.1	5	23.7	0.002	0.159
Motorways>Railways >Water bodies	130766.6	5	25.2	0.005	0.134
Railways>Motorways >Water bodies	130768.4	5	27.0	<0.001	0.156
Distance	130823.1	2	81.7	<0.001	0.007

Table S10: Results of single-surface optimisation (SS_OPTIM()) procedures testing the model support when adding *all vegetation* and *built-up areas* to the three best-supported overlap models in a single-surface analysis (see Table S9). Results are for the Berlin-only dataset, using the FCA-based genetic distance measure. (i), (ii): Initial pre-bootstrapping results. Each optimisation was performed twice for each combination of landscape features; (iii) Bootstrapping results. For each combination of landscape feature, we only included the distance matrix from the run (marked with '^{x}') with the lowest corrected Akaike information criterion (**AIC$_c$**) in the bootstrap analysis. ">" indicates which predictor took priority, meaning that every grid cell containing a point of overlap with other linear predictors, the cell was codified as belonging to the predictor with priority. **avg. AICc** = average of the AICc values obtained for each model in 1000 bootstrap iterations. **ΔAICc** = difference in the (avg.) AICc values between the best supported model and each subsequent model. ***k*** = number of parameters, **m*R*^{2}** = marginal *R*^{2}, **avg.m*R*^{2}** = average marginal *R*^{2} of 1000 bootstrap iterations. **avg.weight** = average of the AICc weights obtained for each model in 1000 bootstrap iterations. **Resistance** = resistance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation). Predictors are sorted according to increasing AIC$_c$ values.

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**	Resistance
(i) first analysis					
Water bodies>Motorways>Railways	232498.4	5	0	0.139	Motorways: 1, Rail: 23, Matrix: 1278, Water: 9376
^{x}Motorways>Water bodies>Railways	232500.7	5	2.3	0.135	Motorways: 1, Rail: 2, Matrix: 192, Water: 1077
^{x}Water bodies>Railways>Motorways	232501.5	5	3.1	0.111	Motorways: 1, Rail: 4, Matrix: 376, Water: 2011
^{x}Motorways>Water bodies>Railways*Vegetation*Built-up areas	232505.6	6	7.2	0.105	Rail: 1, Motorways: 1, Built-up: 121, Vegetation: 146, Water: 498
^{x}Water bodies>Railways>Motorways*Vegetation*Built-up areas	232509.9	6	11.5	0.091	Rail: 1, Motorways: 1, Built-up: 175, Vegetation: 216, Water: 615
Water bodies>Motorways>Railways*Vegetation*Built-up areas	232510.7	6	12.3	0.213	Rail: 1, Motorways: 1, Vegetation: 8, Built-up: 80, Water: 554
Distance	232643.5	2	145.1	0.006	
Null	233094.8	1	596.4	0.000	
(ii) repeat analysis					
^{x}Water bodies>Motorways>Railways*Vegetation*Built-up areas	232493.8	6	0	0.156	Rail: 1, Motorways: 4, Built-up: 291, Vegetation: 494, Water: 1574
^{x}Water bodies>Motorways>Railways	232495.7	5	1.9	0.197	Motorways: 1, Rail: 101, Matrix: 4000, Water: 46145
Motorways>Water bodies>Railways	232500.9	5	7.1	0.150	Motorways: 1, Rail: 3, Matrix: 250, Water: 1605
Water bodies>Railways>Motorways	232504.3	5	10.5	0.093	Motorways: 1, Rail: 8, Matrix: 941, Water: 3398
Motorways>Water bodies>Railways*Vegetation*Built-up areas	232506.9	6	13.1	0.102	Rail: 1, Motorways: 1, Built-up: 156, Vegetation: 199, Water: 500
Water bodies>Railways>Motorways*Vegetation*Built-up areas	232510.9	6	17.1	0.096	Rail: 1, Motorways: 1, Built-up: 234, Vegetation: 328, Water: 678
Distance	232643.5	2	149.7	0.006	
Null	233094.8	1	601	0.000	
Predictor	**avg. AIC$_c$**	***k***	**ΔAIC$_c$**	**avg.weight**	**avg.m*R*^{2}**
(iii) bootstrapping					
Water bodies>Motorways>Railways*Vegetation*Built-up areas	130695.6	6	0	0.463	0.158
Water bodies>Railways>Motorways	130700.2	5	4.5	0.063	0.113
Motorways>Water bodies>Railways	130700.6	5	5.0	0.171	0.136
Motorways>Water bodies>Railways*Vegetation*Built-up areas	130700.9	6	5.3	0.107	0.107
Water bodies>Motorways>Railways	130700.6	5	5.0	0.160	0.197
Water bodies>Railways>Motorways*Vegetation*Built-up areas	130702.6	6	7.0	0.035	0.094
Distance	130781.0	2	85.4	<0.001	0.007

Table S11: Results of single-surface optimisation (SS_OPTIM()) procedure testing whether model support of multi-categorical surfaces is sensitive to the starting values of the input surface. We took multi-categorical surfaces with different combinations of predictors that were retained in the step-wise optimisation procedure (see Tables S8, S9 & S10) and inverted the values of the input surface. We performed a total of four independent optimisation runs for each initial and inverted surface. “>” indicates which predictor took priority, meaning that every grid cell containing a point of overlap with other linear predictors, the cell was codified as belonging to the predictor with priority. Predictors were sorted according to increasing corrected Akaike information criterion (**AIC$_c$**) values. ***k*** = number of parameters, **ΔAICc** = difference in the AICc values between the best-supported model and each subsequent model. **m*R*^{2}** = marginal *R*^{2}. **Resistance** = resistance value inferred for each parameter (matrix= the remaining study).

Predictor	AIC$_c$	*k*	ΔAIC$_c$	m*R*^{2}	Starting values	Resistance
(a)						
Water bodies>Railways	232514.7	4		0.195	Railways: 0, Matrix: 1, Water: 2	Railways: 1, Matrix: 33, Water: 499
Water bodies>Railways	232514.7	4	0	0.193	Railways: 0, Matrix: 1, Water: 2	Railways: 1, Matrix: 33, Water: 499
Water bodies>Railways	232514.9	4	0.2	0.085	Railways: 0, Matrix: 1, Water: 2	Railways: 1, Matrix: 111, Water: 500
Water bodies>Railways	232514.9	4	0.2	0.088	Railways: 0, Matrix: 1, Water: 2	Railways: 1, Matrix: 105, Water: 500
Water bodies>Railways	232635.7	4	121.0	0.029	Water: 0, Matrix: 1, Railways: 2	Water: 1, Matrix: 109, Railways: 215
Water bodies>Railways	232635.7	4	121.0	0.029	Water: 0, Matrix: 1, Railways: 2	Water: 1, Matrix: 109, Railways: 214
Water bodies>Railways	232635.7	4	121.0	0.029	Water: 0, Matrix: 1, Railways: 2	Water: 1, Matrix: 109, Railways: 215
Water bodies>Railways	232635.7	4	121.0	0.029	Water: 0, Matrix: 1, Railways: 2	Water: 1, Matrix: 110, Railways: 215
Distance	232643.5	2	128.8	0.006		
Null	233094.8	1	451.3	0		
(b)						
Water bodies>Motorways>Railways	232495.7	5	0	0.197	Railways: 0, Motorways: 1, Matrix: 2, Water: 3	Motorways: 1, Railways: 101, Matrix: 4000, Water: 46145
Water bodies>Motorways>Railways	232495.7	5	0	0.149	Railways: 0, Motorways: 1, Matrix: 2, Water: 3	Motorways: 1, Railways: 35, Matrix: 2199, Water: 16430
Water bodies>Motorways>Railways	232498.4	5	2.7	0.139	Railways: 0, Motorways: 1, Matrix: 2, Water: 3	Motorways: 1, Railways: 23, Matrix: 1278, Water: 9376
Water bodies>Motorways>Railways	232499.3	5	3.6	0.113	Railways: 0, Motorways: 1, Matrix: 2, Water: 3	Motorways: 1, Railways: 12, Matrix: 1204, Water: 5759
Water bodies>Motorways>Railways	232599.2	5	103.5	0.053	Water: 0, Matrix: 1, Motorways: 2, Railways: 3	Motorways: 1, Water: 87, Matrix: 4022, Railways: 13346
Water bodies>Motorways>Railways	232601.0	5	105.3	0.063	Water: 0, Matrix: 1, Motorways: 2, Railways: 3	Motorways: 1, Water: 63, Matrix: 4143, Railways: 14586
Water bodies>Motorways>Railways	232601.0	5	105.3	0.041	Water: 0, Matrix: 1, Motorways: 2, Railways: 3	Motorways: 1, Water: 40, Matrix: 2406, Railways: 4582
Water bodies>Motorways>Railways	232608.0	5	112.3	0.089	Water: 0, Matrix: 1, Motorways: 2, Railways: 3	Motorways: 1, Water: 11, Matrix: 1038, Railways: 4906
Distance	232643.5	2	147.8	0.006		
Null	233094.8	1	599.1	0		
(c)						
Water>Motorw.>Rail*All vege.*Built-up areas	232490.9	6	0	0.215	Rail.: 0, Motor.: 1, Vege.: 2, Built-up: 3, Water: 4	Rail.: 1, Motorw.: 7, All vege.: 294, Built-up: 251, Water: 2349
Water>Motorw.>Rail*All vege.*Built-up areas	232493.8	6	2.9	0.156	Rail.: 0, Motor.: 1, Vege.: 2, Built-up: 3, Water: 4	Rail.: 1, Motorw.: 4, Built-up: 291, All vege.: 494, Water: 1574
Water>Motorw.>Rail*All vege.*Built-up areas	232505.7	6	14.8	0.109	Rail.: 0, Motor.: 1, Vege.: 2, Built-up: 3, Water: 4	Rail.: 1, Motorw.: 2, Built-up: 307, All vege.: 357, Water: 829
Water>Motorw.>Rail*All vege.*Built-up areas	232510.7	6	19.8	0.213	Rail.: 0, Motor.: 1, Vege.: 2, Built-up: 3, Water: 4	Rail.: 1, Motorw.: 1, All vege.: 8, Built-up: 80, Water: 554
Water>Motorw.>Rail*All vege.*Built-up areas	232600.6	6	109.7	0.057	Water: 0, Built-up: 1, Vege.: 2, Motor.: 3, Rail.: 4	Motorw.: 1, Water: 129, All vege.: 5011, Built-up: 6201, Rail.: 20351
Water>Motorw.>Rail*All vege.*Built-up areas	232601.5	6	110.6	0.049	Water: 0, Built-up: 1, Vege.: 2, Motor.: 3, Rail.: 4	Motorw.: 1, Water: 109, All vege.: 5791, Built-up: 6661, Rail.: 16167
Water>Motorw.>Rail*All vege.*Built-up areas	232606.1	6	115.2	0.054	Water: 0, Built-up: 1, Vege.: 2, Motor.: 3, Rail.: 4	Motorw.: 1, Water: 27, All vege.: 2039, Built-up: 2688, Rail.: 5587
Water>Motorw.>Rail*All vege.*Built-up areas	232606.4	6	115.5	0.080	Water: 0, Built-up: 1, Vege.: 2, Motor.: 3, Rail.: 4	Motorw.: 1, Water: 29, Built-up: 2118, All vege.: 2494, Rail.: 9811
Distance	232643.5	2	152.6	0.006		
Null	233094.8	1	603.9	0		

Table S12: Initial results of the multi-categorical functional connectivity analysis for the city of Berlin. Individual predictors were added based on corrected Akaike information criterion (**AICc**) model support, but only retaining if their addition improved support of the multi-categorical model (ΔAICc>2; see Materials and Methods). Presented here are the results from the two initial (first & repeat analysis) optimisation runs that were performed for each (combination of) landscape features. Given previous results (Table S10), *water bodies* took precedence over *motorways* at points of overlap, while both took precedence of *railways*. Predictors were sorted according to increasing AIC$_c$ values. ***k*** = number of parameters, **ΔAICc** = difference in the AICc values between the best-supported model and each subsequent model. **m*R***^{2} = marginal R^2. **Resistance** = resistance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation).

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R***^{2}	Resistance
(i) first analysis					
^{x}Water bodies*Railways*Motorways*S.L.30-50%*S.L.>80%	232473.8	7	0	0.189	Rail: 1, S.L.30-50%: 1, Motorways: 3, Matrix: 254, S.L.>80%: 555, Water: 1185
^{x}Water*Railways*Motorways*S.L.30-50%*S.L.50-80%*S.L.>80% *Remaining built-up	232475.9	9	2.1	0.151	Rail: 1, Motorways: 1, S.L.30-50%: 35, S.L.50-80%: 58, Remaining built-up: 259, Matrix: 284, S.L.>80%: 394, Water: 527
Water bodies*Railways*Motorways*S.L.30-50%	232479.3	6	5.5	0.168	Rail: 1, Motorways: 3, S.L.30-50%: 19, Matrix: 234, Water: 1214
Water*Railways*Motorways*S.L.30-50%*S.L.>80% *Industry	232481.7	8	7.9	0.166	Motorways: 1, Rail: 2, S.L.30-50%: 9, Matrix: 290, S.L.>80%: 807, Industry: 849, Water: 947
Water*Railways*Motorways*S.L.30-50%*S.L.50-80%*S.L.>80%	232484.2	8	10.4	0.139	Rail: 1, Motorways: 2, S.L.50-80%: 84, Matrix: 271, S.L.30-50%: 304, S.L.>80%: 685, Water: 727
^{x}Water bodies*Railways*Motorways*Arable/green*S.L.30-50%	232489.7	7	15.9	0.223	Rail: 1, Motorways: 1, S.L.30-50%: 3, Arable/green: 12, Matrix: 166, Water: 650
Water*Railways*Motorways*S.L.30-50%*S.L.>80% *Remaining built-up	232497.5	8	23.7	0.217	Rail: 1, Motorways: 2, Matrix: 113, S.L.30-50%: 125, Remaining built-up: 427, Water: 833, S.L.>80%: 986
Water bodies*Railways *Motorways	232498.4	5	24.6	0.139	Motorways: 1, Rail: 23, Matrix: 1278, Water: 9376
Water bodies*Railways *Motorways*Arable/green	232499.8	6	26.0	0.153	Rail: 1, Motorways: 3, Matrix: 258, Arable/green: 649, Water: 1049
Water*Railways*Motorways*S.L.30-50%*S.L.50-80%*S.L.>80%*Industry	232500.2	9	26.4	0.106	Rail: 1, Motorways: 1, S.L.50-80%: 91, S.L.30-50%: 230, Matrix: 237, S.L.>80%: 323, Industry: 398, Water: 419
Water*Railways*Motorways*All vegetation*Built-up areas	232510.7	6	36.9	0.213	Rail: 1, Motorways: 1, All vegetation : 8, Built-up areas: 80, Water: 554
Water bodies*Railways	232514.9	4	41.1	0.085	Rail: 1, Matrix: 111, Water: 500
^{x}Water bodies	232528.8	3	55.0	0.303	Matrix: 1, Water: 44
^{x}Railways	232577.5	3	103.7	0.055	Rail: 1, Matrix: 99
Motorways	232593.0	3	119.2	0.014	Motorways: 1, Matrix: 500
^{x}Arable/Green	232601.4	3	127.6	0.048	Arable/Green: 1, Matrix: 67
^{x}S.L.30-50%	232606.3	3	132.5	0.016	S.L.30%-50%: 1, Matrix: 647
^{x}S.L.>80%	232617.4	3	143.6	0.050	Matrix: 1, S.L.>80%: 8
^{x}All vegetation	232625.9	3	152.1	0.029	Vegetation: 1, Matrix: 9
S.L.50-80%	232629.1	3	155.3	0.035	S.L.50%-80: 1, Matrix: 140
^{x}Built up areas	232632.2	3	158.4	0.028	Matrix: 1, Built-up: 6
^{x}Industry	232632.9	3	159.1	0.029	Matrix: 1, Industry: 6
^{x}Remaining built-up	232640.5	3	166.7	0.022	Matrix: 1, Remaining built-up: 7
Distance	232643.5	2	169.7	0.006	
^{x}Forest	232644.9	3	171.1	0.009	Matrix: 1, Forest: 2
Null	233094.8	1	621.0	0.000	

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**	Resistance
(ii) repeat analysis					
^{x}Water*Railways*Motorways*S.L.30-50%*S.L.50-80%*S.L.>80%*Industry	232473.4	9	0	0.201	Rail: 1, Motorways: 3, S.L.30-50%: 43, S.L.50-80%: 142, Matrix: 292, Industry: 452, S.L.>80%: 633, Water: 1756
^{x}Water*Railways*Motorways*S.L.30-50%*S.L.50-80%*S.L.>80%	232473.7	8	0.3	0.169	Rail: 1, Motorways: 2, S.L.30-50%: 8, S.L.50-80%: 103, Matrix: 282, S.L.>80%: 469, Water: 784
^{x}Water bodies*Railways*Motorways*S.L.30-50%	232474.9	6	1.5	0.179	Rail: 1, Motorways: 3, S.L.30-50%: 3, Matrix: 278, Water: 1196
^{x}Water*Railways*Motorways*S.L.30-50%*S.L.>80% *Industry	232478.6	8	5.2	0.185	Motorways: 1, Rail: 51, S.L.30-50%: 1033, Matrix: 3848, Industry: 12803, S.L.>80%: 21019, Water: 24576
Water*Railways*Motorways*S.L.30-50%*S.L.50-80%*S.L.>80% *Remaining built-up	232484.3	9	10.9	0.114	Rail: 1, Motorways: 1, S.L.30-50%: 111, Matrix: 143, S.L.50-80%: 147, Remaining built-up: 319, Water: 456, S.L.>80%: 459
^{x}Water*Railways*Motorways*S.L.30-50%*S.L.>80% *Remaining built-up	232485.5	8	12.1	0.149	Rail: 1, Motorways: 2, S.L.30-50%: 59, Matrix: 165, Remaining built-up: 166, S.L.>80%: 545, Water: 795
^{x}Water*Railways*Motorways*All vegetation*Built-up areas	232493.8	6	20.4	0.156	Rail: 1, Motorways: 4, Built-up areas: 291, All vegetation : 494, Water: 1574
^{x}Water bodies*Railways*Motorways	232495.7	5	22.3	0.197	Motorways: 1, Rail: 101, Matrix: 4000, Water: 46145
^{x}Water bodies*Railways *Motorways*Arable/green	232498.7	6	25.3	0.111	Rail: 1, Motorways: 2, Matrix: 193, Arable/green: 237, Water: 790
Water bodies*Railways*Motorways*Arable/green*S.L.30-50%	232511.2	7	37.8	0.123	Motorways: 1, Rail: 2, S.L.30-50%: 5, Arable/green: 102, Matrix: 313, Water: 385
^{x}Water bodies*Railways	232514.9	4	41.5	0.088	Rail: 1, Matrix: 105, Water: 500
Water bodies*Railways*Motorways*S.L.30-50%*S.L.>80%	232527.8	7	54.4	0.298	Motorways: 1, Rail: 1, Matrix: 305, S.L.30-50%: 400, Water: 416, S.L.>80%: 451
Water bodies	232528.8	3	55.4	0.303	Matrix: 1, Water: 44
Railways	232577.5	3	104.1	0.055	Rail: 1, Matrix: 99
^{x}Motorways	232592.9	3	119.5	0.014	Motorways: 1, Matrix: 500
Arable/Green	232601.4	3	128.0	0.048	Arable/Green: 1, Matrix: 69
S.L.30%-50%	232613.7	3	140.3	0.013	S.L.30%-50%: 1, Matrix: 29
S.L.>80%	232617.4	3	144.0	0.050	Matrix: 1, S.L.>80%: 8
All vegetation	232626.0	3	152.6	0.026	Vegetation: 1, Matrix: 8
^{x}S.L.50%-80%	232629.1	3	155.7	0.037	S.L.50%-80: 1, Matrix: 217
Built-up areas	232632.2	3	158.8	0.028	Matrix: 1, Built-up: 7
Industry	232632.9	3	159.5	0.029	Matrix: 1, Industry: 6
Remaining built-up	232640.5	3	167.1	0.022	Matrix: 1, Remaining built-up: 7
Distance	232643.5	2	170.1	0.006	
Forest	232644.9	3	171.5	0.009	Matrix: 1, Forest: 2
Null	233094.8	1	621.4	0.000	

Table S13: Results of single-surface optimisation (SS_OPTIM()) procedures testing the effect the Berlin city border on gene flow in Berlin/Brandenburg (the complete data set). The single-feature analysis tested for the effect of the administrative city border, the administrative city border converted into a concave hull, as well as the outer and inner borders of 1-, 2-, 3, 4- and 5-km buffer around the concave hull. (i), (ii): Results from two initial optimisation runs performed for each landscape feature; (iii) Bootstrapping results. Since corrected Akaike information criterion (**AIC$_c$**) values were equal between the two optimisation runs, we used the results from the first run in the bootstrap analysis. Predictors are sorted according to increasing AIC$_c$ values. **ΔAICc** = difference in the (avg.) AICc values between the best supported model and each subsequent model. ***k*** = number of parameters, **m*R*^{2}** = marginal *R*^{2}, **avg.m*R*^{2}** = average marginal *R*^{2} of 1000 bootstrap iterations. **avg.weight** = average of the AICc weights obtained for each model in 1000 bootstrap iterations. **Resistance** = resistance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation).

Predictor	**AIC$_c$**	***k***	**ΔAIC$_c$**	**m*R*^{2}**	**Resistance**
(i) first analysis					
City border concave	555283.5	3	0	0.416	Matrix: 1, Border: 154
City border concave + 1 km	555346.7	3	63.2	0.337	Matrix: 1, Border: 160
City border concave + 2 km	555349.2	3	65.8	0.279	Matrix: 1, Border: 183
City border concave + 3 km	555414.2	3	130.7	0.229	Matrix: 1, Border: 147
City border	555435.9	3	152.5	0.268	Matrix: 1, Border: 92
City border concave + 5 km	555461.1	3	177.7	0.194	Matrix: 1, Border: 124
City border concave + 4 km	555473.3	3	189.9	0.146	Matrix: 1, Border: 119
City border concave - 1 km	555477.1	3	193.6	0.211	Matrix: 1, Border: 63
City border concave - 5 km	555488.5	3	205.0	0.086	Matrix: 1, Border: 43
City border concave - 4 km	555499.7	3	216.2	0.076	Matrix: 1, Border: 40
City border concave - 3 km	555507.7	3	224.3	0.112	Matrix: 1, Border: 33
City border concave - 2 km	555509.7	3	226.2	0.128	Matrix: 1, Border: 42
Distance	555549.5	2	266.0	0.011	
Null	556351.0	1	1067.6	0	
(ii) repeat analysis					
City border concave	555283.5	3	0	0.416	Matrix: 1, Border: 154
City border concave + 1 km	555346.7	3	63.2	0.337	Matrix: 1, Border: 160
City border concave + 2 km	555349.2	3	65.8	0.279	Matrix: 1, Border: 183
City border concave + 3 km	555414.2	3	130.7	0.229	Matrix: 1, Border: 147
City border	555435.9	3	152.5	0.268	Matrix: 1, Border: 92
City border concave + 5 km	555461.1	3	177.7	0.194	Matrix: 1, Border: 124
City border concave + 4 km	555473.3	3	189.9	0.146	Matrix: 1, Border: 120
City border concave - 1 km	555477.1	3	193.6	0.211	Matrix: 1, Border:
City border concave - 5 km	555488.5	3	205.0	0.086	Matrix: 1, Border: 43
City border concave - 4 km	555499.7	3	216.2	0.076	Matrix: 1, Border: 40
City border concave - 3 km	555507.7	3	224.3	0.112	Matrix: 1, Border: 33
City border concave - 2 km	555509.7	3	226.2	0.128	Matrix: 1, Border: 42
Distance	555549.6	2	266.1	0.011	
Null	556351.0	1	1067.6	0	
Predictor	**avg. AIC$_c$**	***k***	**ΔAIC$_c$**	**avg.weight**	**avg.m*R*^{2}**
(iii) bootstrapping					
City border concave	310808.4	3	0	0.699	0.400
City border concave + 1 km	310858.9	3	50.5	0.107	0.315
City border concave + 2 km	310859.8	3	51.4	0.156	0.237
City border concave + 3 km	310895.0	3	86.6	0.021	0.200
City border	310909.7	3	101.3	<0.001	0.255
City border concave + 5 km	310926.1	3	113.6	0.005	0.182
City border concave - 1 km	310925.5	3	117.7	0.003	0.209
City border concave + 4 km	310932.3	3	117.1	0.004	0.138
City border concave - 5 km	310936.7	3	123.9	0.005	0.087
City border concave - 4 km	310943.0	3	128.3	0.001	0.077
City border concave - 3 km	310945.4	3	134.6	<0.001	0.113
City border concave - 2 km	310961.5	3	137.0	<0.001	0.129
Distance	310926.1	2	153.1	<0.001	0.012

Table S14: Initial pre-bootstrapping results of the single-feature ResistanceGA analysis for the complete dataset. Three different genetic distance measures and seven environmental predictors from the German authoritative topographic cartographic information system (ATKIS) were compared (see Material and Methods). To check for convergence, optimisation was performed twice for each landscape feature (Run 1 & Run 2). Predictors are sorted according to increasing average corrected Akaike information criterion (AIC_c) values. ***k*** = number of parameters, **ΔAIC_c** = difference in the AIC_c values between the best supported model (lowest AIC_c) and each subsequent model. **mR^2** = marginal R^2. **Resistance** resistance/conductance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation).

Predictor	**AIC_c**	***k***	**ΔAIC_c**	**mR^2**	**Resistance**		**AIC_c**	***k***	**ΔAIC_c**	**mR^2**	**Resistance**	
					Matrix	**Predictor**					**Matrix**	**Predictor**
a) Nei's genetic distance (Nei), run 1							run 2					
Built-up areas	-98031.1	3	0	0.024	1	2	-98031.1	3		0.024		2
Forests	-98024.0	3	7.1	0.037	6	1	-98024.0	3		0.037	6	1
Arable/green	-98014.5	3	9.5	0.026	3	1	-98014.5	3		0.026	3	1
Motorways	-98000.6	3	13.9	0.075	452	1	-98000.6	3		0.075	452	1
City border concave	-97993.9	3	6.7	0.045	1	10	-97993.9	3		0.045	1	10
Water bodies	-97988.5	3	5.4	0.040	1	3	-97988.5	3		0.040	1	3
Railways	-97988.3	3	0.2	0.029	1	2	-97988.3	3		0.029	1	2
Distance	-97982.8	2	5.5	0.034			-97982.8	2		0.034		
Null	-97496.7	1	486.1	0.000			-97496.7	1		0.000		
b) Proportion of shared alleles (D_{PS}) , run 1							run 2					
Built-up areas	-97533.6	3	0	0.020	1	2	-97533.6	3		0.020		2
Forests	-97523.2	3	10.4	0.029	4	1	-97523.2	3		0.029	4	1
Arable/green	-97522.1	3	1.1	0.023	3	1	-97522.1	3		0.023	3	1
Motorways	-97510.3	3	11.8	0.069	354	1	-97510.3	3		0.069	354	1
City border concave	-97502.1	3	8.2	0.037	1	7	-97502.1	3		0.037		7
Railways	-97500.2	3	1.9	0.029	1	2	-97500.2	3		0.029		2
Water bodies	-97498.4	3	1.8	0.035	1	2	-97498.4	3		0.035		2
Distance	-97496.8	2	1.6	0.033			-97496.8	2		0.033		
Null	-97033.3	1	463.5	0.000			-97033.3	1		0.000		
c) 10-axes based Factorial Correspondence Analysis (FCA) , run 1							run 2					
City border concave	555283.5	3	0	0.416	1	154	555283.5	3		0.416	1	154
Built-up areas	555345.4	3	61.9	0.056	1	7	555345.4	3		0.056	1	7
Forests	555368.1	3	22.7	0.051	500	1	555368.1	3		0.051	500	1
Arable/green	555404.3	3	36.2	0.036	16	1	555404.3	3		0.036	16	1
Water bodies	555412.1	3	7.8	0.171	1	31	555412.1	3		0.171	1	31
Railways	555478.8	3	66.7	0.063	1	14	555478.8	3		0.063	1	14
Distance	555549.5	2	70.7	0.011			555549.5	2		0.011		
Motorways	555552.0	3	2.5	0.025	388	1	555551.4	3		0.011	1	1
Null	556351.0	1	799.0	0.000			556351.0	1		0.000		

Table S15: Initial results of the multi-categorical functional connectivity analysis for the complete dataset (Berlin & Brandenburg). Individual predictors were added based on corrected Akaike information criterion (**AICc**) model support, but only retaining if their addition improved support of the multi-categorical model (ΔAICc>2; see Materials and Methods). Presented here are the results from the two initial (first & repeat analysis) optimisation runs that were performed for each (combination of) landscape features. We also tested two overlap scenarios, once where *water bodies* took precedence over *railways* at points of overlap (Water bodies>Railways), and another where the opposite was the case (Railways >Water bodies). Predictors were sorted according to increasing AIC_c values. ***k*** = number of parameters, **ΔAICc** = difference in the AICc values between the best-supported model and each subsequent model. **m*R*^{2}** = marginal R^2. **Resistance** = resistance value inferred for each parameter (matrix= the remaining uniform study area not containing the features under investigation).

Predictor	AIC_c	*k*	ΔAIC_c	mR^2	Resistance
(ii) first analysis					
City border concave* Built-up areas*Water bodies	554938.3	5	0	0.400	Matrix: 1, Built-up areas: 6, Water bodies: 70, City border: 498
City border concave *Built-up areas *Water bodies>Railways	554939.3	6	1.0	0.390	Railways: 1, Matrix: 2, Built-up areas: 19, Water bodies: 109, City border: 760
City border concave *Built-up areas * Railways >Water bodies	554964.1	6	25.8	0.451	Matrix: 1, Railways: 4, Built-up areas: 48, Water bodies: 186, City border: 1615
^{x}City border concave* Built-up areas	555017.3	4	79.0	0.425	Matrix: 1, Built-up areas: 11, City border: 499
^{x}City border concave* Built-up areas * Arable/green	555018.1	5	79.8	0.520	Arable/green: 1, Matrix: 6, Built-up areas: 34, City border: 2358
^{x}City border concave* Built-up areas *Forests	555038.2	5	99.9	0.359	Matrix: 1, Forest: 1, Built-up areas: 16, City border: 486
^{x}City border concave	555283.5		345.2		Matrix: 1, City border: 154
^{x}Built-up areas	555345.4	3	407.1	0.056	Matrix: 1, Built-up areas: 7
^{x}Forests	555368.1	3	429.8	0.051	Forests: 1, Matrix: 500
^{x}Arable/green	555404.3	3	466.0	0.036	Arable/Green: 1, Matrix: 16
^{x}Water bodies	555412.1	3	473.8	0.171	Matrix: 1, Water bodies: 31
^{x}Railways	555478.8	3	540.5	0.063	Matrix: 1, Rail: 14
Distance	555549.5	2	611.2	0.011	
^{x}Motorways	555552.0	3	613.7	0.025	Motorways: 1, Matrix: 388
Null	556351.0	1	1412.7	0	
(ii) repeat analysis					
City border concave* Built-up areas*Water bodies	554938.0	5	0	0.402	Matrix: 1, Built-up areas: 6, Water bodies: 72, City border: 500
City border concave *Built-up areas *Water bodies>Rail	554940.3	6	2.3	0.417	Railways: 1, Matrix: 2, Built-up areas: 13, Water bodies: 108, City border: 736
City border concave *Built-up areas *Rail>Water bodies	554965.7	6	27.7	0.475	Matrix: 1, Railways: 3, Built-up areas: 26, Water bodies: 155, City border: 1274
City border concave* Built-up areas	555017.3	4	79.3	0.425	Matrix : 1, Built-up areas :11, City border : 500
City border concave* Built-up areas *Forests	555080.9	5	142.9	0.324	Matrix: 1, Forest: 2, Built-up areas: 47, City border: 984
City border concave* Built-up areas * Arable/green	555135.1	5	197.1	0.194	Matrix: 1, Arable/green: 6, Built-up areas: 21, City border: 434
City border concave	555283.5	3	345.5		Matrix: 1, City border: 154
Built-up areas	555345.4	3	407.4	0.056	Matrix : 1, Built-up areas : 7
Forests	555368.1	3	430.1	0.051	Forests: 1, Matrix: 500
Arable/green	555404.3	3	466.3	0.036	Arable/Green: 1, Matrix: 16
Water bodies	555412.1	3	474.1	0.171	Matrix: 1, Water bodies: 31
Railways	555478.8	3	540.8	0.063	Matrix: 1, Rail: 14
Distance	555549.5	2	611.5	0.011	
Motorways	555554.1	3	616.1	0.011	Motorways: 1, Matrix: 1
Null	556351.0	1	1413.0	0	

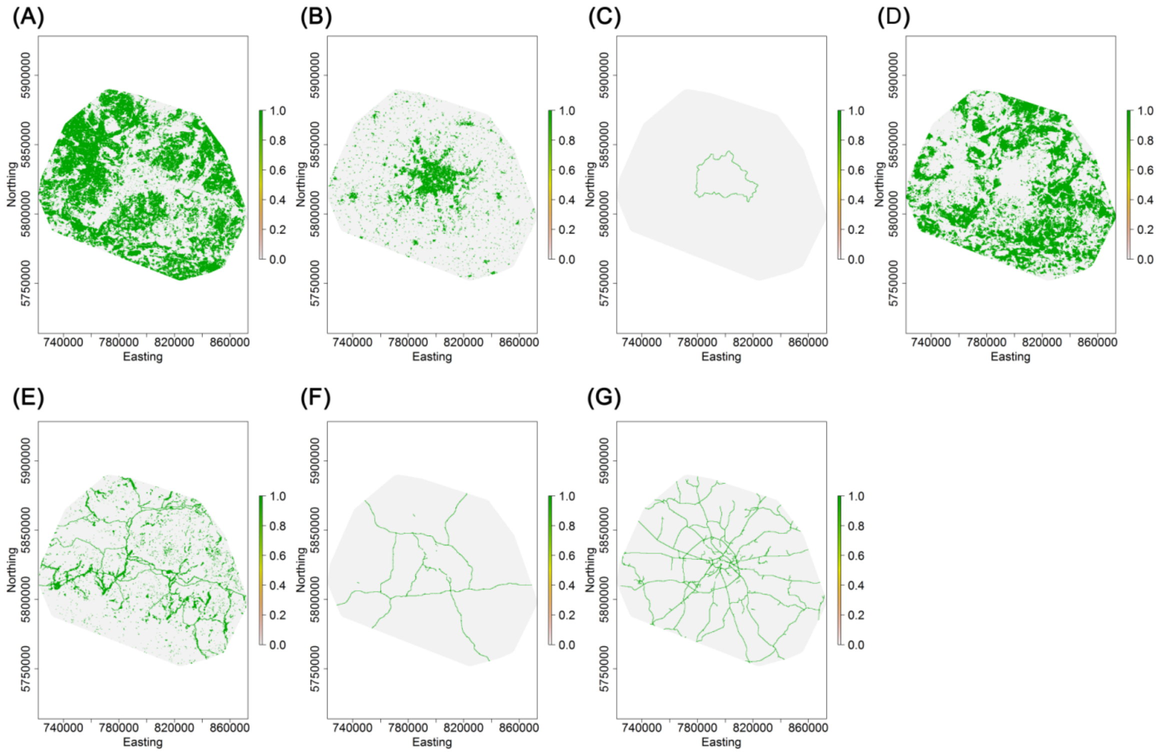

Fig. S1: Single-predictor input rasters for the whole study area (Berlin & Brandenburg) based on the ATKIS data. (A) *arable/green*, (B) *built-up areas*, (C) *concave city border*, (D) *forests*, (E) major *water bodies*, (F) *motorways* and (G) *railways*. The green surface represents the predictor under investigation. The grid cell size was 250 x 250 m.

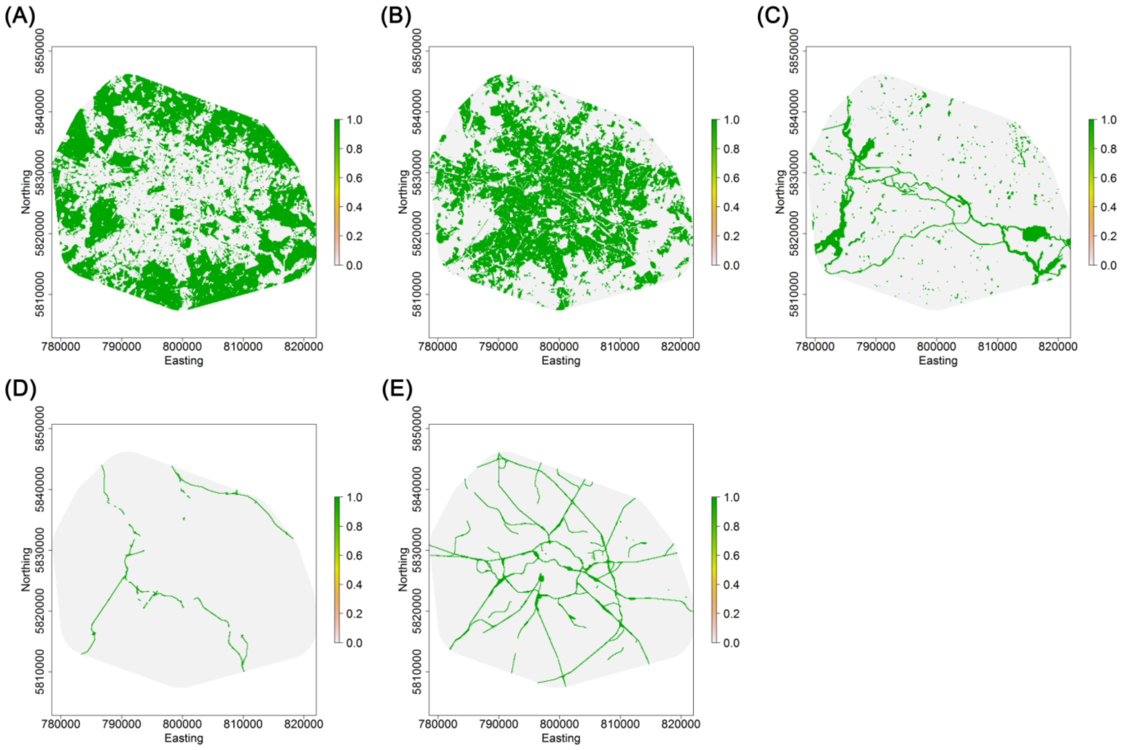

Fig. S2: Single-predictor input rasters for the city of Berlin based on the ATKIS data. (A) *all vegetation*, (B) *built-up areas*, (C) major *water bodies*, (D) *motorways* and (E) *railways*. The green surface represents the predictor under investigation. The grid cell size was 100 x 100 m.

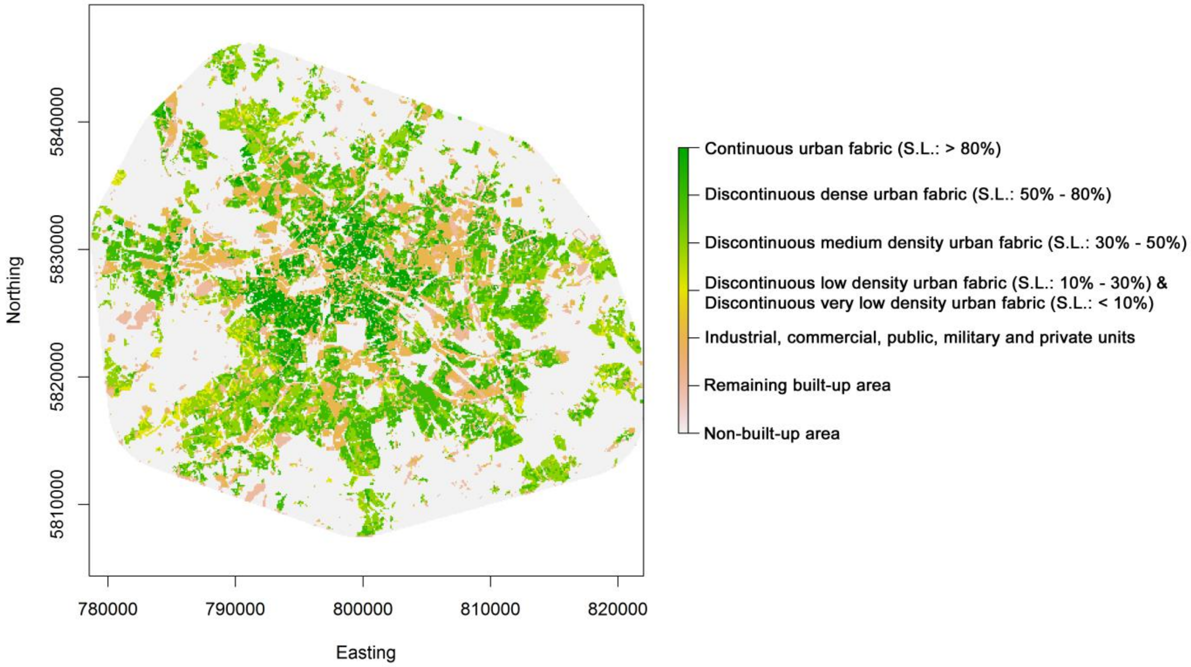

Fig. S3: Geographic distribution of the Copernicus Urban Atlas imperviousness land cover types that were located in the built-up areas and used in the present analysis. Due to the rare occurrence of discontinuous very low density urban fabric and remaining built-up areas, the corresponding categories were merged with discontinuous low density urban fabric (the created the *remaining built-up areas* predictor). The data are represented in the form of the 100 x 100 m grid used for analysis.

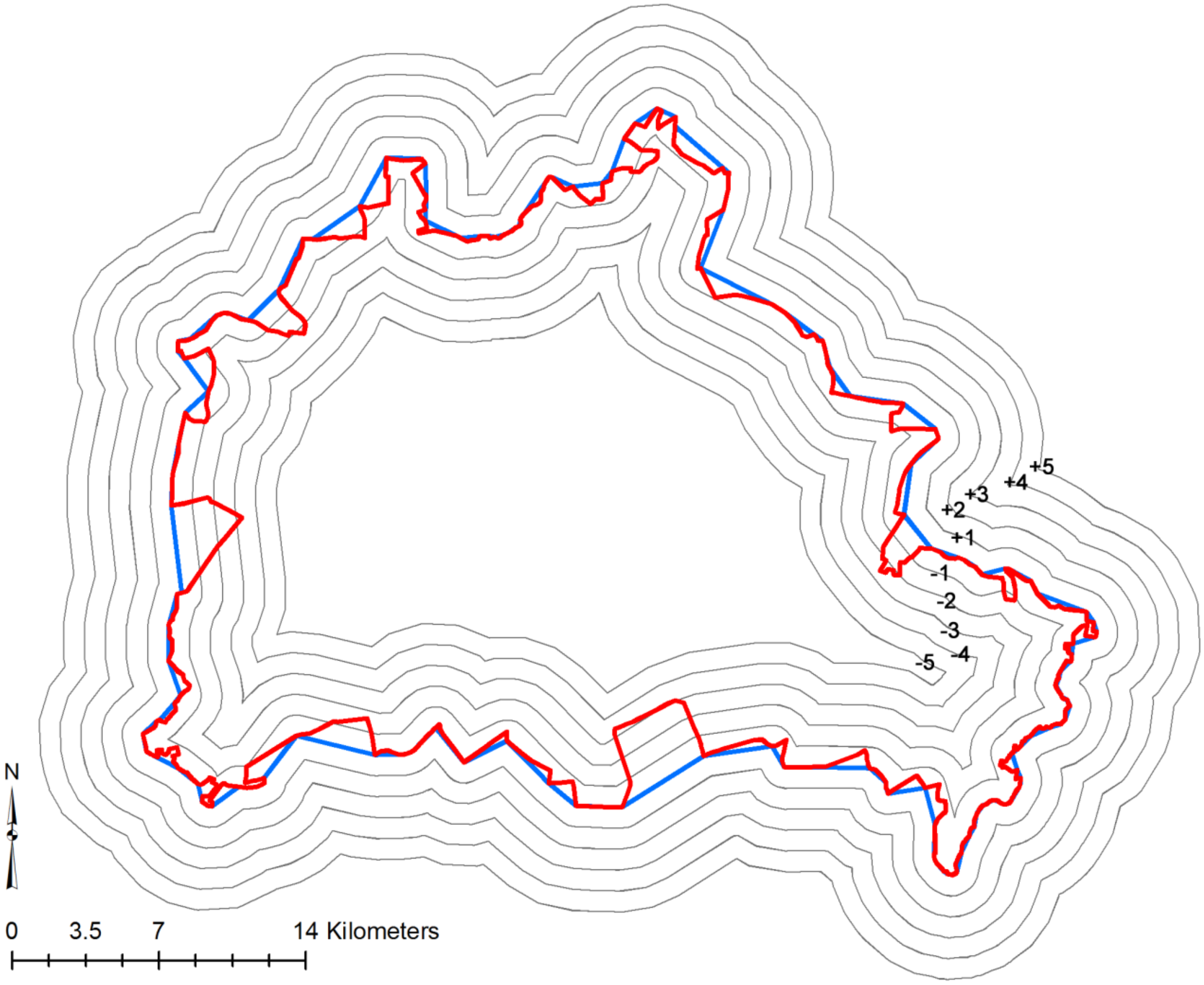

Fig. S4: Different line shapes used to test for the effect of the city border on gene flow. The red line represents the administrative border of the city; the blue line the administrative city border converted into a concave hull and the remaining lines the inner and outer border of a 1-, 2-, 3-, 4- and 5-km buffer around the concave hull.

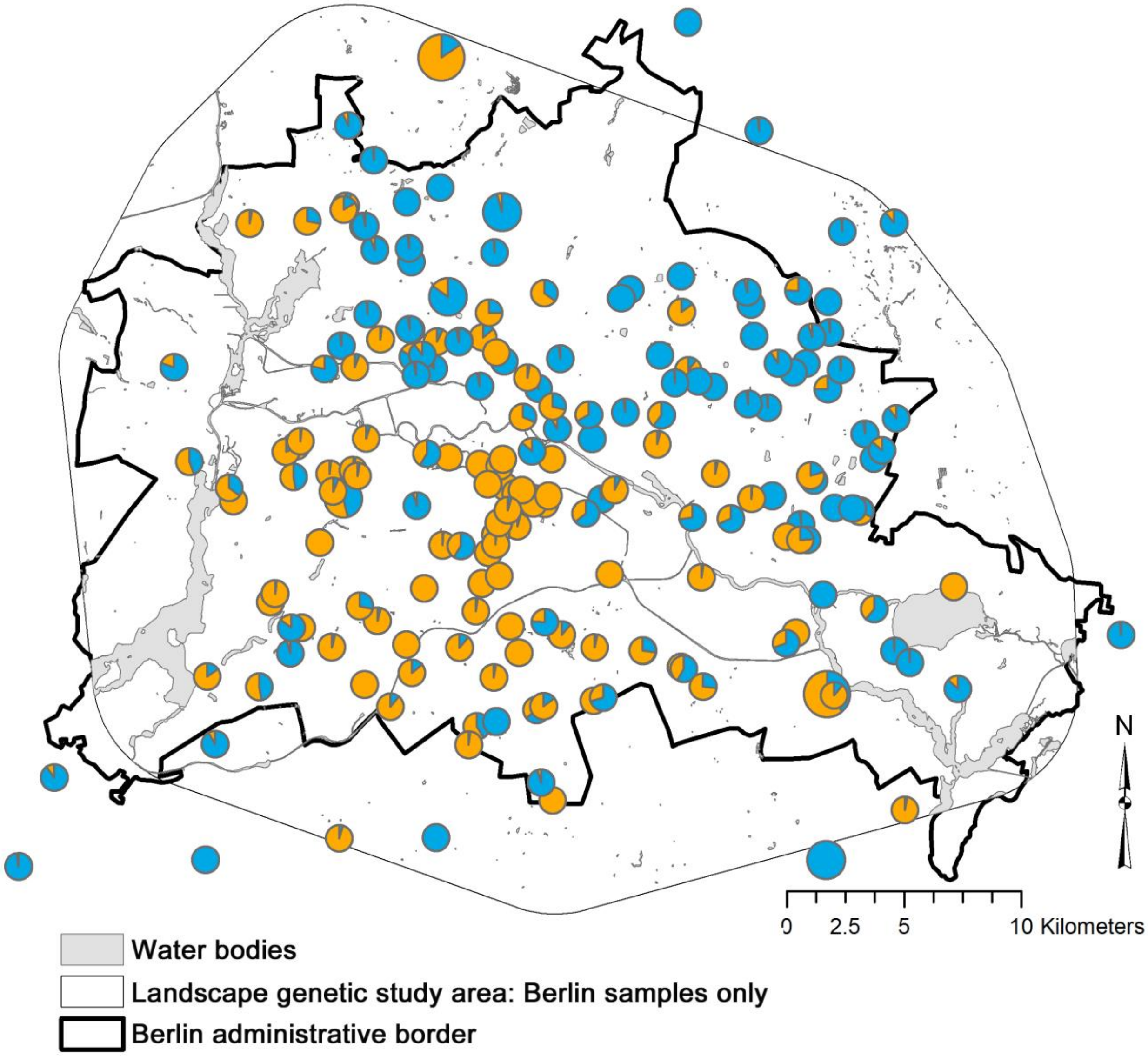

Fig. S5: Clustering results of STRUCTURE for the city of Berlin, including water bodies and city border. The size of the pie charts represents the number of samples per spot; the colours represent the percentage assignment to the detected clusters.

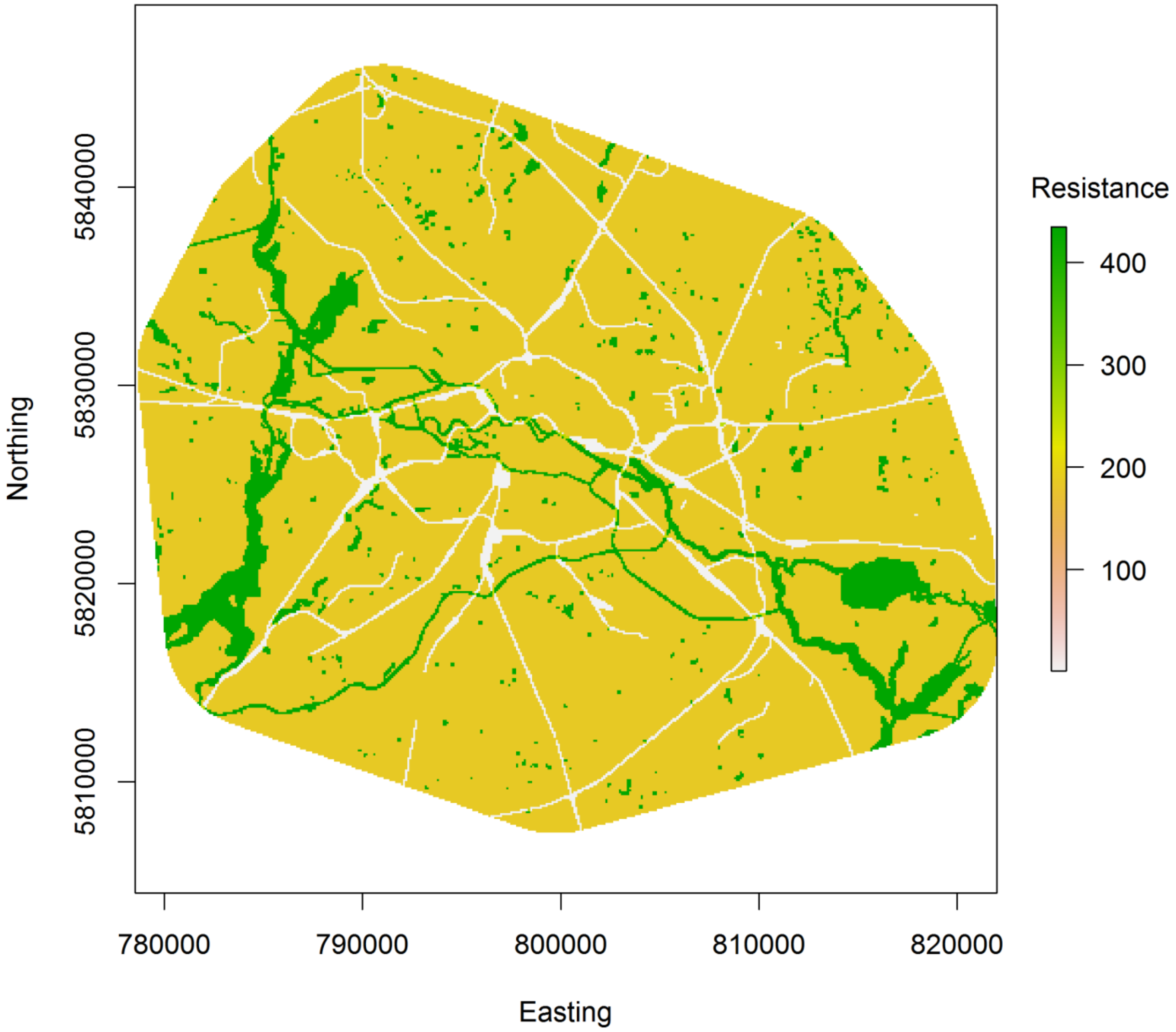

Fig. S6: Best-supported resistance model (*water bodies* & *railways*) resulting from the optimisation of all possible combination of the five single ATKIS environmental predictors (ALL_COMB() function). The surface has a distinct resistance value for areas where *water bodies* and *railways* overlap.

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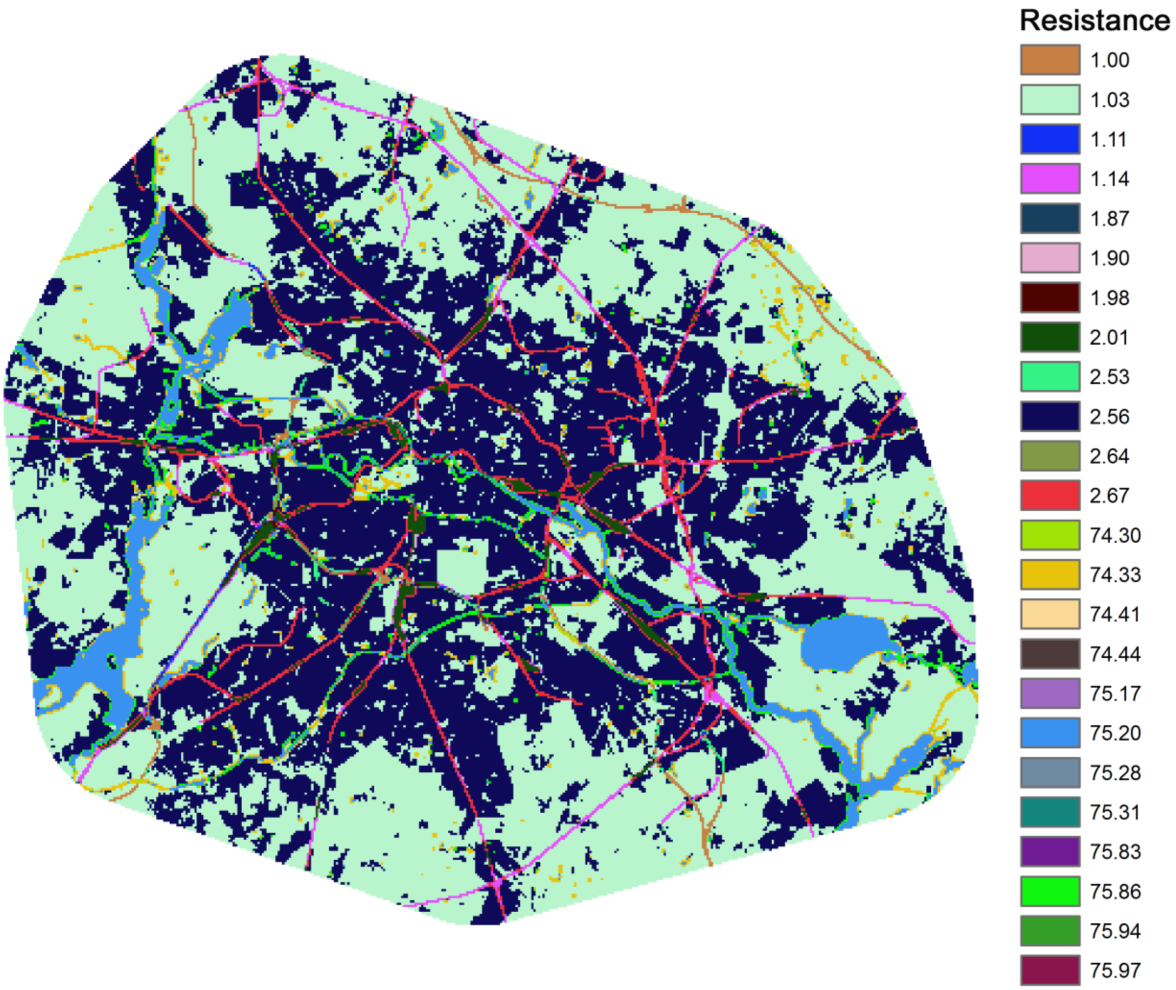

Fig. S7: Resistance values generated for the model containing all five ATKIS environmental predictors during the analysis where possible combination of the five single ATKIS environmental predictors were optimised using the ALL_COMB() function. ResistanceGA gave different resistance values to the linear features depending on with which other feature they overlapped with. For geographic scale, please refer to Fig. S5.

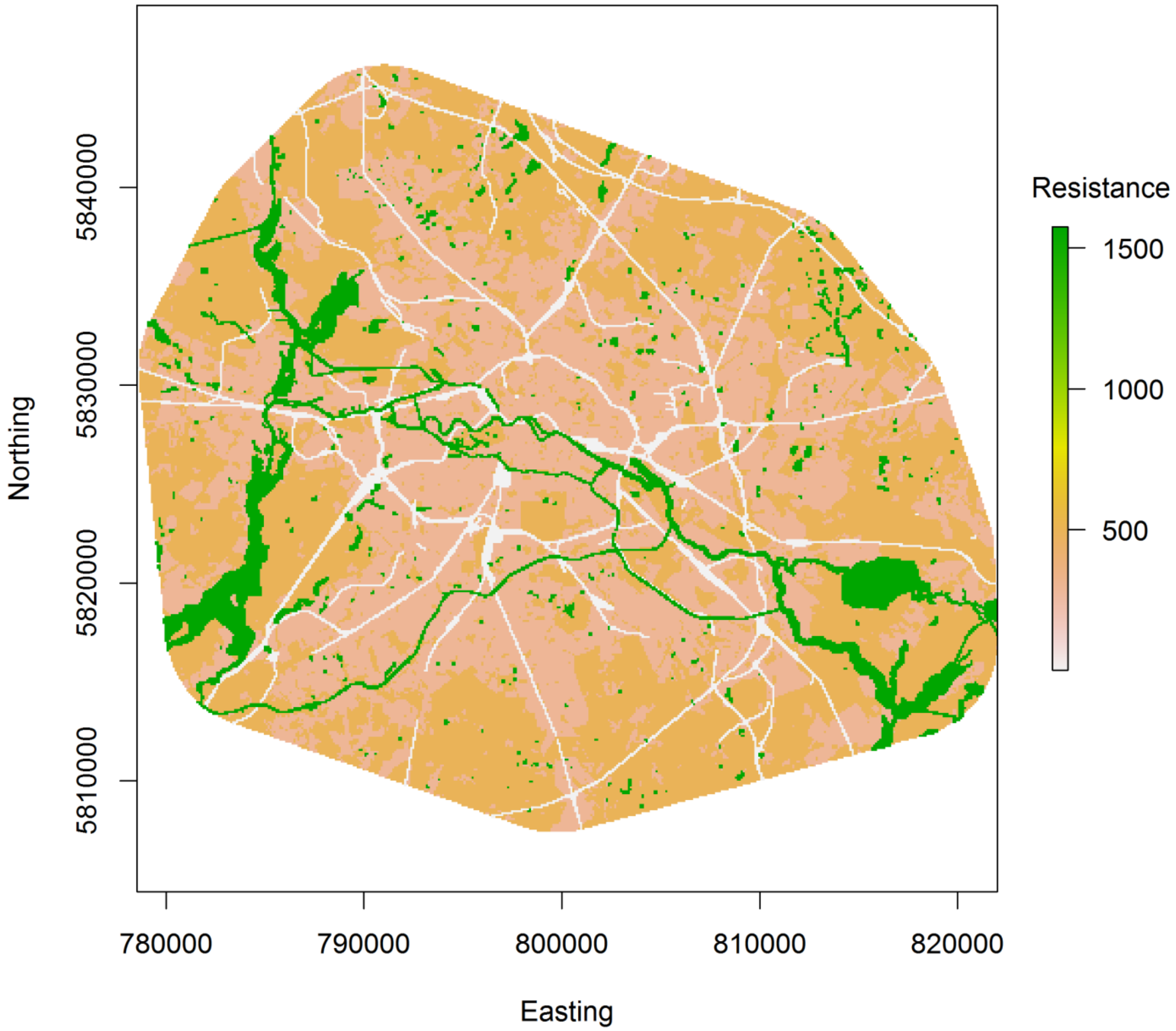

Fig. S8: Best-supported multi-categorical resistance surface for Berlin based on ATKIS data, resulting from single-surface optimisation (SS_OPTIM()) procedure, where the individual environmental features were added and optimised step-by-step based on the model support of the individual features. At points of overlap, w*ater bodies* took precedence over *motorways* and *railways* in the resistance grid, while *motorways* took precedence over *railways*.

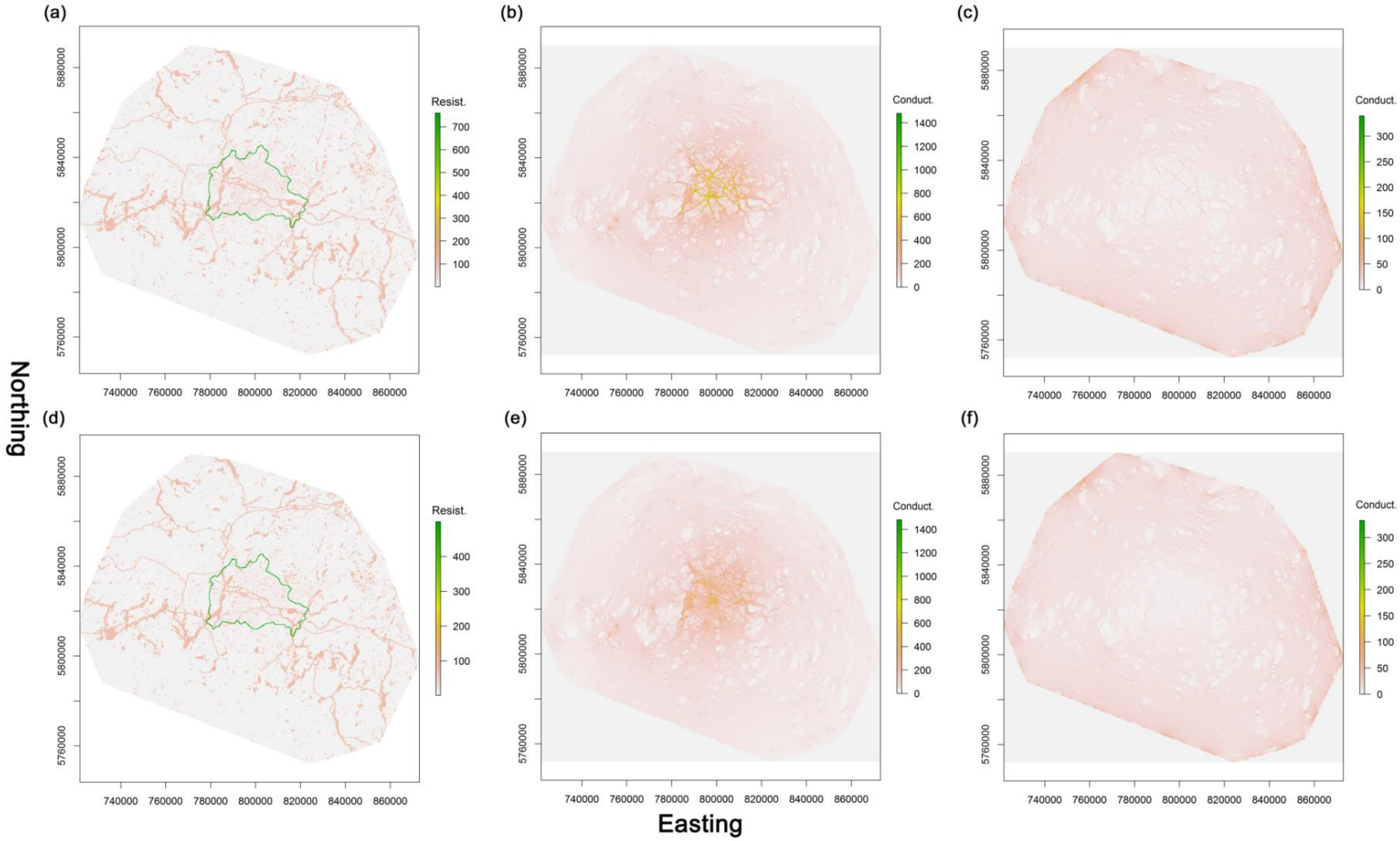

Fig. S9: Cartographic representation of results from genetics-based resistance modelling for foxes across the whole study area. (a) Optimised resistance surface of the overall best multi-categorical model and the corresponding CIRCUITSCAPE connectivity map showing conductance to gene flow based on (b) the sample locations and (c) sampling locations simulated at the edge of the study area. (d) Optimised resistance surface of the second-best-supported multi-categorical model ($\Delta AICc<2$) and the corresponding CIRCUITSCAPE connectivity map showing conductance to gene flow based on (e) the sample locations and (f) sampling locations simulated at the edge of the study area.

Chapter 2: Spatial ecology of urban red foxes

The limits of serenity - avoidance behaviour towards humans in an opportunistic predator

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Author contribution

The original idea to study red fox space use and movement patterns was developed by Heribert Hofer (HH), Miriam Brandt (MB) and Stephanie Kramer-Schadt (SKS). The detailed concept and the focus on habitat selection in response to landscape and human presence was elaborated by Sophia E. Kimmig (SEK). The methodological approach was chosen by Aimara Planillo (AP), HH and SEK. Organisation and conduction of field work was done by SEK. Trapping, radio collaring and tracking was done by SEK and supported by Konstantin Börner (KB). SEK selected covariates and reclassified landscape features. AP and Moritz Wenzler-Meya (MWM) contributed to the generation of raster layers. MWM and SEK did the data preparation. SEK conducted data analysis, supported by AP and MWM. SEK wrote the manuscript. HH, MB and SKS contributed with substantial revisions to the manuscript.

The limits of serenity - avoidance behaviour towards humans in an opportunistic predator

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2.1 Abstract

Urban areas test the boundaries of the capacities of wildlife species to adjust to novel environments. The red fox (*Vulpes vulpes*) is one of several carnivores considered to be an opportunistic generalists which successfully inhabits urban areas worldwide. This success predicts that red foxes are expected to adjust to the urban environment as well as to actual human presence and activity. Yet, today's carnivores in urban landscapes descended from usually shy and elusive rural ancestors subjected to intense hunting pressure by humans for centuries. This suggests that the behavioural adjustment of the urban dwelling animals to human activities has its limits and that human presence *per se* should in general be avoided. The classification of red foxes and other carnivores as "urban dwellers" or "urban adapters" does not separate adjustment to anthropogenic structures and environments from adjustments to human activities. To address this issue we analysed high resolution movements of red foxes in the conurbation of Berlin, Germany, across a variety of urban habitats which differed in the density of the built-up area and the degree to which human activity was present. We generated available habitat selection points based on movement patterns and characterised locations available to and used by foxes by assessing covariates associated with landscape classes, degree of urbanisation and human presence. These were analysed with a step selection function and a generalised linear mixed model framework. The results indicated that foxes preferred locations with low human presence such as abandoned areas and railways and avoided public green spaces and city forests exposed to intensive human use. Areas of high human population density were avoided by foxes, irrespective of the ubiquitous presence of foxes throughout the city area, whereas artificial environments such as built-up areas were not. We conclude that actual human presence and activities set stronger limits to the animals' life history than anthropogenic structures, even in established urban commensals such as the red fox.

Keywords: habitat selection, landscape of fear, spatial ecology, urban ecology

2.2 Introduction

Understanding how animals use space and chose habitats is a fundamental issue in ecology (Gaillard et al. 2010). An animal's use of space and choice of habitats emerge from its movement patterns (Karelus et al. 2019). The underlying behavioural strategies may depend on intrinsic as well as extrinsic conditions such as climate, predation risks (Raynor et al. 2017, Heithaus 2001), resource dispersion or resource competition (Carr & Macdonald 1986, Rosenzweig 1991). Thus habitat selection and movement patterns may document micro evolutionary strategies in behaviour on an individual scale and reflect macro evolutionary patterns of adaptation (Morris 2003).

The behavioural strategies of animals largely depend on the availability of habitat types (Mysterud & Ims 1998). Urban areas provide a variety of different habitat types, ranging from highly built-up areas and areas of high percentages of sealed ground to green, vegetated patches in parks, cemeteries or remnants of natural areas. Depending on their degree of synanthropy (Fischer et al. 2015) urban wildlife species may prefer or avoid different urban sites. As naturally shy and elusive species (Macdonald 1978, Mahon et al. 1989) red foxes (*Vulpes vulpes*) might prefer patches within urban environments that are similar to natural-like habitats present in the rural environment of their ancestors. However, as established human commensal species, their inherent plasticity (Macdonald 1978) may enable behavioural adjustments by red foxes to urban living, resulting in an even use of both natural and built-up habitats without specific preferences. Consistent with this idea, one study on habitat selection by red foxes found that they did not consistently select a particular habitat (Cavallini & Lovari 1994). Urban landscapes are not only characterised by artificial structures, sealed surfaces or man-made barriers but also by human presence and activities. The presence of people in different city areas and, more indirectly, the local density of the human population may modify fox habitat use patterns. Consistent with this idea, a recent camera trap study showed that red fox activity patterns were determined by several interacting drivers (Diaz-Ruiz et al. 2015). Diaz-Ruiz et al. (2015) suggested to investigate the importance of human presence in greater detail as fox activity rhythms seemed to be determined by human presence in sites where human disturbance was high. More recently, a study on landscape resistance to gene flow across the urban-rural gradient demonstrated that human presence may be a key driver of fox dispersal patterns (Kimmig et al. 2020).

In other words, foxes might or might not respond to anthropogenic influences on the landscape, in terms of the degree of built-up area and anthropogenic structures, and/or they might respond to human presence and activity as such. These ideas and results suggest three hypotheses on which specific element of potential anthropogenic disturbance red foxes might respond most strongly to. Hypothesis (i) suggests that the foxes' behavioural plasticity and adjustment to urban environments enables them to fully exploit the urban area. This predicts that foxes should evenly use the urban landscape, no specific landscape elements are especially avoided, and human population density should not have an impact on red fox movements. Hypothesis (ii) suggests that even in urban environments red foxes rely on green, vegetated areas. This predicts that they avoid built-up areas and grey spaces, specifically select green spaces, including public green spaces and forested areas, whilst human population density is of no relevance. Hypothesis (iii) suggests that red foxes adjust their space use to human presence, i.e. foxes should avoid habitats associated with human presence. This predicts that foxes should avoid densely populated areas and prefer sites inaccessible to humans. If foxes avoid human presence and activities, their preference of undisturbed habitat (such as wasteland) should be more distinct at times of overall increased human activity.

In this study we used GPS movement data to assess which source of anthropogenic disturbance affects red fox movement and space use behaviour in urban environments. This included a comparison of (movement) habitat selection during key human activity times with habitat selection at times of reduced or no human activity.

2.3 Materials & Methods

Study area and trapping

The Berlin metropolitan area in the Northeast of Germany (52.5200° N, 13.4050° E, Fig. 1a) is characterised by a high level of heterogeneity. The city, populated by 3.5 million inhabitants, includes highly industrialised and densely built-up parts with fully sealed surfaces as well as spacious forested areas. Around 40% of the 900km² city area are covered by forest (~18%) and other green spaces (~12%), agriculture (~4%) and water (~7%). The built-up areas are interspersed by around 2,500 public green spaces, 75,000 allotment gardens, 220 cemeteries and numerous waste land sites with a total of around 11,000ha (GRIS 2021).

We used animal-borne global positioning system (GPS) data of sixteen wild red foxes that were captured within the Berlin city area between 2015 and 2018 (Fig. 1b, Fig. S1). Foxes were trapped at multiple locations, using wooden live traps of two meter length (Weisser, trap number 0580) with wire trigger. At capturing sites, traps were set, regularly baited with dog food and left open for several months. Camera traps were used to verify fox visits at the traps to decide when traps should be armed. During armed periods, traps were monitored through electronic trap supervision which sent an alert once the trapdoors were closed (Trapmaster, EPV Electronics GmbH) and traps were also checked on a regular basis.

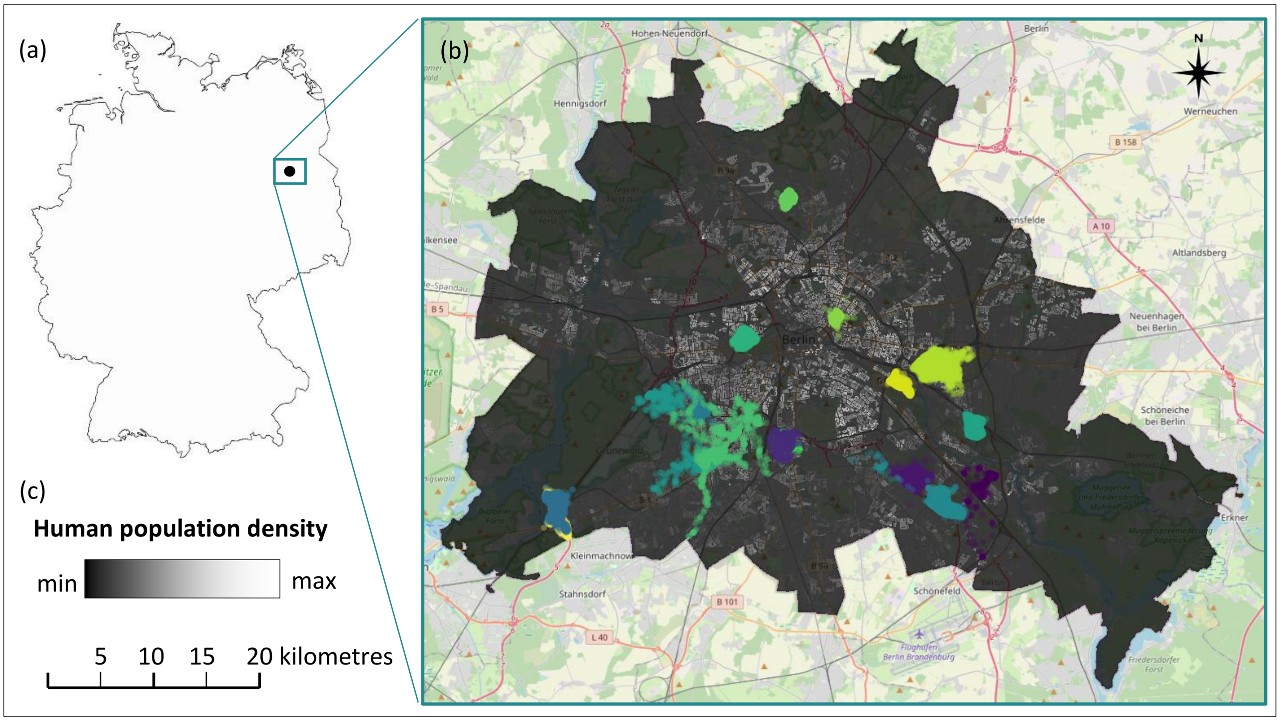

Figure 1: a: Location of the study area within Germany. b: The city area of Berlin (map: OSM) with the human population density raster and GPS localizations of the radio collared foxes. c: legend referring to panel b.

The ensuing handling of trapped foxes was conducted at the trapping location. Animal handling permits were approved by the respective animal welfare licensing committee of Berlin ("Landesamt für Gesundheit und Soziales", permit number: G 0211/15). Foxes were first transferred to a crush cage where they were weighed and their general physical condition was checked to define the appropriate dosage of analgesics. Only foxes of a good weight (>4.5kg) and good health were included in the study. They were anaesthetised using a combination of medetomedine (0.07mg/kg) and midazolam (0.8mg/kg). Drugs were administered by intramuscular injection in the quadriceps or gluteal muscles. During anaesthesia, the face of the foxes was covered with a towel and the eyes treated with a gel to prevent the cornea from drying out. During the entire a procedures a veterinarian regularly checked vital signs, pulse and oxygen saturation were monitored with a non-invasive pulse oximetry device, with the sensor attached to the tongue of the fox. All foxes were deployed with VHF-ACC-GPS collars (e-obs®, Grünwald, models 1C-heavy (~180g) or 1C-light (~110g)). At the end of the procedure foxes were placed in the trap on a bedding of hay where they woke up and from where they could leave the trap on their own and at a time of their own choosing. The raw data taken by the radio collars were stored on-board. During a daily one hour time frame a VHF signal was emitted that could be detected by a handheld receiver to locate the fox and the data downloaded in the field, using the handheld e-obs base station.

Data and analysis

GPS locations (fixes) were sampled every four or every twenty minutes, with some foxes starting at a four minute sampling interval which was later reduced to twenty minutes. Additionally, acceleration data (ACC) were sampled every two minutes. In order to extend battery lifetime, GPS sampling intervals were reduced to four hours during times of inactivity as defined by a programmed ACC threshold (ACC informed GPS-downregulation) and reactivated and set back to the original interval as soon as the foxes moved again. In order to accommodate ACC-informed GPS downregulation during inactivity of the foxes, we filled the resulting gaps in the data stream with the fox's last fix. Fixes with odd time stamps, locations outside the study area and locations resulting in highly irregular speeds between points were considered technical outliers and excluded from the curated dataset. Gaps originating from such tag errors or tag malfunction remained in the data set. In order to make the data comparable between all individuals, we resampled the fixes to twenty minutes intervals. Raw GPS fixes and individual information are stored at movebank.org and can be shared upon request.

Environmental data were extracted at each fix using package `amt` (Signer et al. 2019) in R. All environmental data originated from the cartographic information service "Geoportal Berlin (FIS broker)" and are available for download. The human population density values were extracted from the polygon "Einwohnerdichte 2019", the percentage degree of sealed surface (imperviousness) from the dataset "Flächennutzung, Stadtstruktur 2015 und Versiegelung 2016". The landscape classification was based on the land use categories of the "Reale Nutzung 2015 (Umweltatlas)". All layers were converted into raster with 10 x 10m resolution. We used the following seven landscape categories reclassified from the original land use data set: (i) *Public green spaces* – city parks and publicly accessible green areas, (ii) *City forest* – forested areas within Berlin irrespective of their composition, (iii) *Wasteland* – abandoned areas and fenced areas such as building land, industrial building remains and construction sites, including fenced verges of railway lines, (iv) *Built-up areas* – residential, industrial and commercial areas of medium dense or dense housing, (v) *Housing with gardens* – residential houses with gardens and sparse housing surrounded by green areas, (vi) *Allotment gardens* – small rental gardens, usually a mixture of small buildings and green spaces and arranged in colonies, and (vii) *Grey spaces* – streets, places and squares.

We used the step selection function (SSF) in the `amt` package to generate ten alternative locations for each fix in each step, to assess ‘available’ habitat. SSF are suitable to identify fine-scale behavioural responses of animals to their environment as they provide an objective method for defining habitat availability in terms of movement constraints (Fieberg et al. 2021, Fortin et al., 2005) and were developed to deal with serial dependence (Thurfjell et al., 2014). So, rather than treating fixes as independent events, step-selection functions model animal tracks, under consideration of serial appearance (the sequence of fixes), step length and turning angles. The `amt` package provides spatial modelling functions too but is designed to assess habitat selection by each individual. As behaviour may vary between animals depending on sex, age, body condition, life history stage or personality (Lesmerises & St-Laurent 2017, Leclerc et al. 2016, Stamps 2007), we built a generalised linear mixed model using the `glmmTMB` package (Magnusson et al. 2017) to assess general habitat selection patterns across individuals and account for inter-individual differences (see also Muff et al. 2019).

In order to test for differences between diurnal and nocturnal behaviour of the foxes in relation to human activity patterns, we run three different land use models: one for data recorded during daytime (from sunrise to dusk), one for nocturnal data (from dusk to sunrise) and a global one with data for all 24 hours. In order to resemble human activity times and patterns more closely, “daytime” includes also fixes measured during dusk whereas “night-time” also included fixes during morning twilight. We also run single models for imperviousness and human population density and compared the latter for diurnal and nocturnal fixes. Finally we ran a full model, including all land use categories as well as human population density and imperviousness using both the full data set and a movement data set which only included fixes when foxes were active (with a minimum step length of 20m between points) and therefore excluded longer resting events. Environmental continuous variables were scaled and centred to aid model convergence. The used (1) versus available (0) fixes were used as response variable. Fox identity and step identity were included as random intercepts in the models. Following Muff et al. (2019), random slopes per individual for the explanatory variables were also included in the model, using a Poisson error distribution.

2.4 Results

All sixteen radio collared foxes were adult with most individuals being in their second year of life and few older individuals. Seven male and nine female foxes were tracked for up to 403 days (Fig. S1). The most common cause for the end of the deployment was mortality from road traffic. Home range sizes substantially varied between individuals (from 14 ha to 200 ha, 95% kernel), as did diurnal space use patterns (see Table S1 and Fig. S2). The mean distance between two consecutive GPS points was 56m in the full dataset and 137m when only fixes of active movement were included. During times of activity (with 19% of movement fixes recorded between sunrise and sunset) foxes moved similar mean distances during day (152m), night (133m) and twilight (154m). The mean distance moved during dawn (112m) was smaller than during dusk (166m).

Habitat selection

The degree of imperviousness of areas played no role in habitat selection (generalised linear mixed model [glmm], parameter estimate β = 0.04 ± 0.04, p = 0.374, Fig. 2a). The glmm for human population density (β = -0.19 ± 0.06, p = 0.002) showed significant avoidance of high human population densities, so foxes preferably used areas with low human population densities (Fig. 2b).

The glmm for the selection probability of land use classes showed positive selection values for wasteland, residential houses with gardens and built-up areas and negative selection for allotment gardens, public green spaces and city forests in relation to grey spaces as reference class (Fig. 3), with significant positive selection for wasteland ($\beta_{wasteland}$ = 0.54 ± 0.27, p = 0.045) and significant negative selection for forest (β_{forest} = -0.88 ± 0.45, p = 0.049). The complete model output is reported in Table S2.

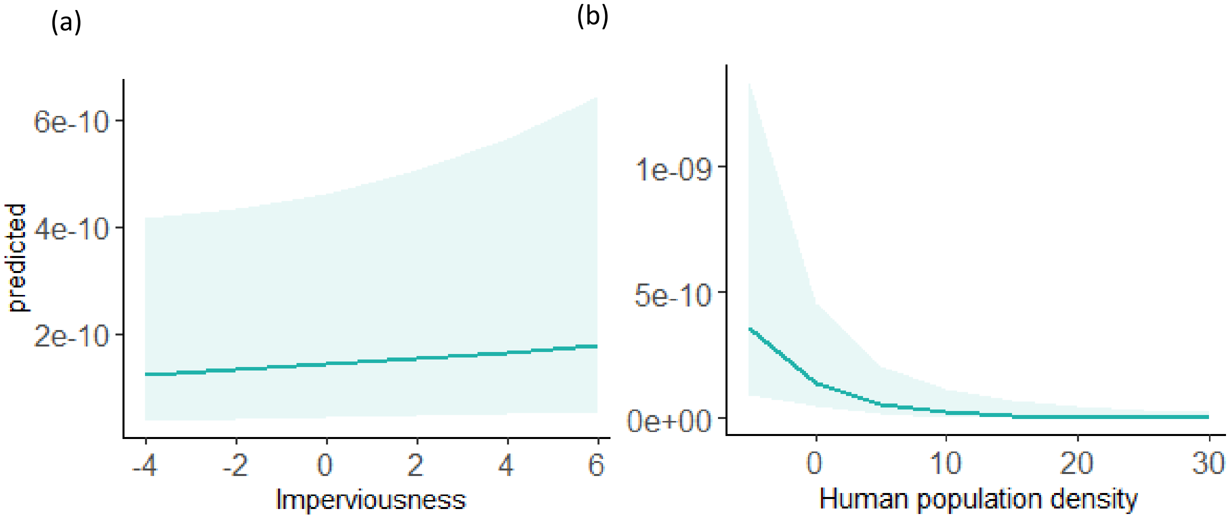

Figure 2: a: The percentage degree of imperviousness of the study area had no significant effect on the selection probability by the study animals. b: Increasing human population density decreased selection probability.

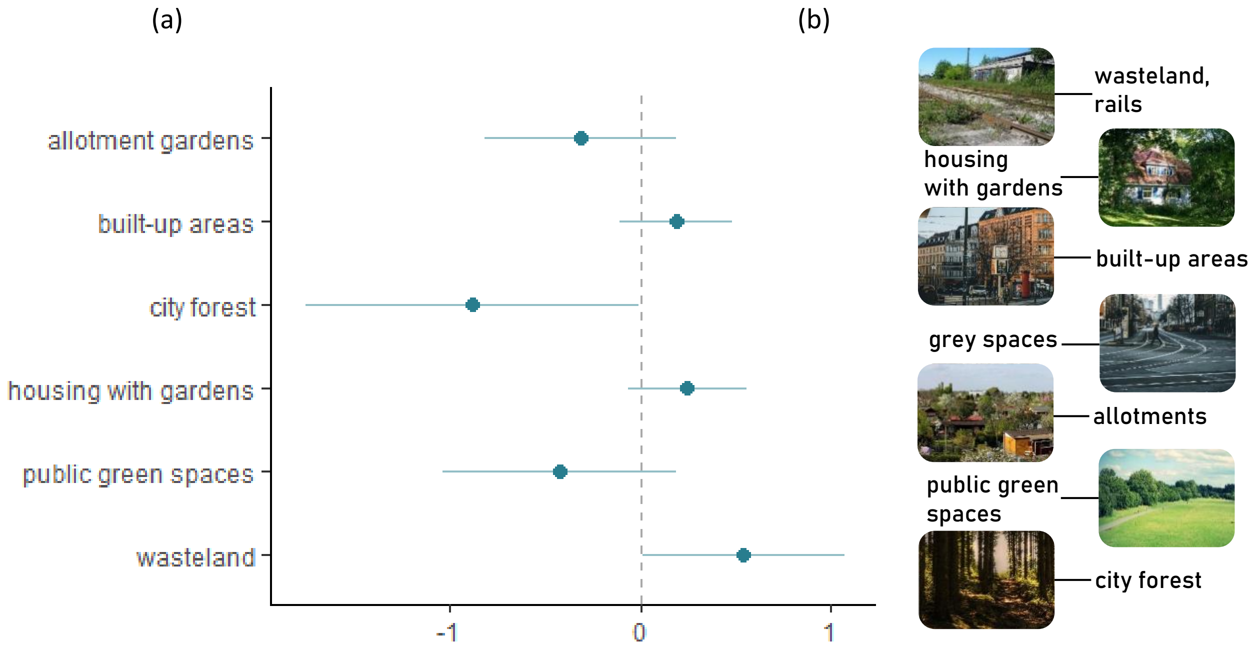

Figure 3: (a) Selection of different land scape types. Positive values indicate preference, negative values avoidance of land scape types in relation to the reference category grey spaces (indicated by the dashed line). (b) Order of selection of land use types from most (top) to least selected (bottom).

Diurnal effects

Modelling habitat selection separately for the daytime and the night-time dataset showed that wasteland, residential houses with gardens and built-up areas show positive, public green spaces, allotment gardens and forests negative selection values in relation to grey spaces as a reference value (Fig. 4). Wasteland was preferentially selected during daytime (1.17 ± 0.42, $p < 0.005$) in relation to grey spaces as a references value. The complete model output is in the supplementary material table S3 (daytime) and table S4 (night-time). High human population density values were significantly avoided during both periods, daytime ($\beta = -0.36 \pm 0.15$, $p = 0.018$) and night-time ($\beta = -0.19 \pm 0.06$, $p = 0.007$).

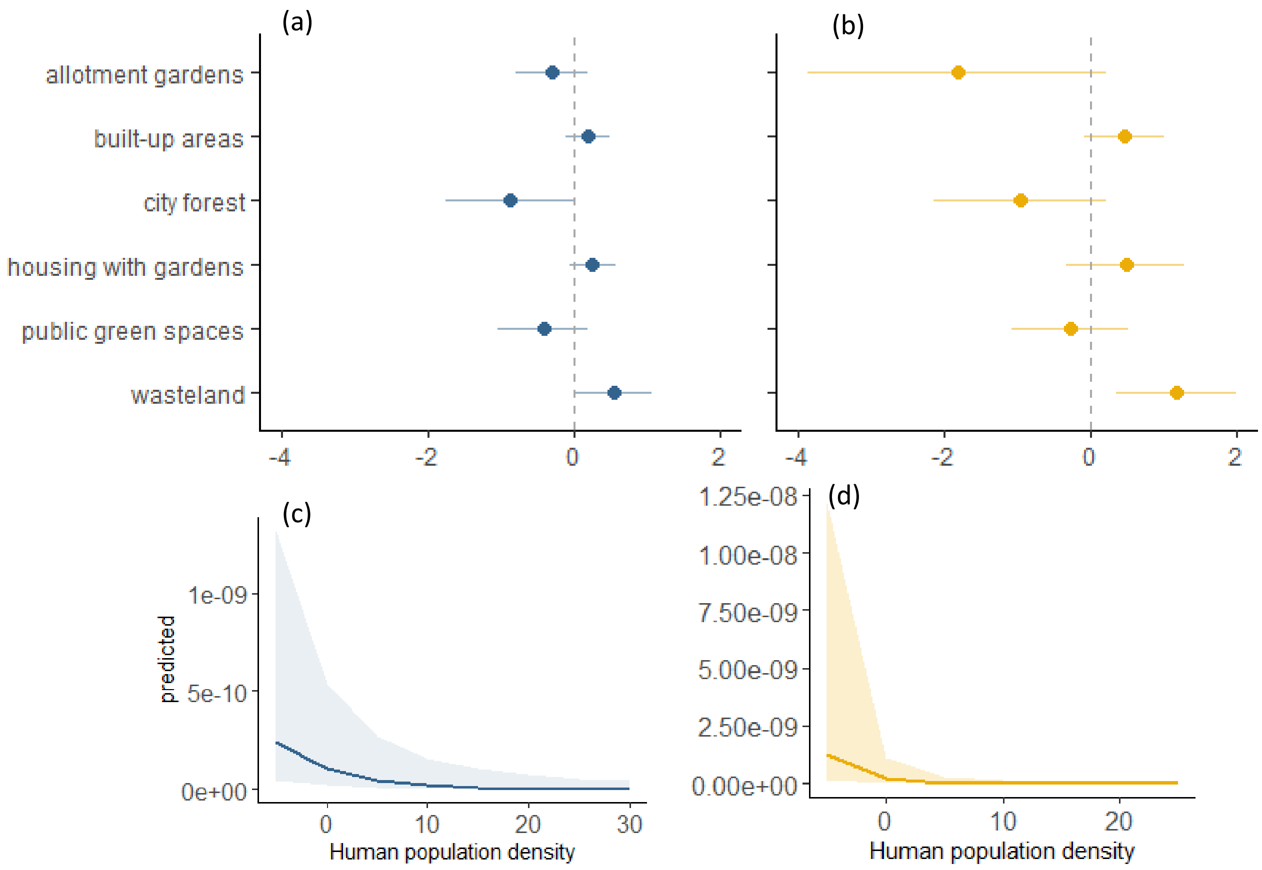

Figure 4: Selection of different landscape types during night-time (a) and daytime (b). Positive values indicate preference, negative values avoidance of landscape types in relation to the reference category grey spaces (indicated by the dashed line). Selection probability in relation to human population density during night-time (c) and during daytime (d). Higher human population densities are significantly less selected.

Space use and movement habitat selection – full model

According to the full model, including all land use covariates, population density and imperviousness (Fig. 5a), high human population densities ($\beta_{\text{population density}}$ =-0.12 ± 0.05, p = 0.011) and imperviousness ($\beta_{\text{imperviousness}}$ = -0.15 ± 0.05, p = 0.004) were significantly avoided. Forests were significantly avoided (β_{forest} = -0.90 ± 0.46, $p < 0.05$), residential houses with gardens ($\beta_{\text{housing with gardens}}$ = 0.56 ± 0.19, p = 0.03), built-up areas ($\beta_{\text{built-up areas}}$ = 0.51 ± 0.18, p = 0.006) and wasteland ($\beta_{\text{wasteland}}$ = 0.77 ± 0.25, p = 0.002) were significantly preferred (see Table S5 for all values). The order of selection probability regarding land use types is equivalent to the pure land use model (see Fig. 3b). It is important to note that all covariates may be relevant to the biological interpretation as significance values depend on the selected reference value (e.g. if wasteland is set as a reference value, *all* other covariates are significantly avoided [Fig. S3, Table S6]).

Fig. 5b shows the model predictions, using only points of active movement only, to analyse movement habitat use excluding resting behaviour. According to the model output city forests (β_{forest} = -0.77 ± 0.33, p = 0.020) as well as allotment gardens ($\beta_{allotment\ gardens}$ = -0.61 ± 0.24, p = 0.013) are significantly avoided (complete output in table S7).

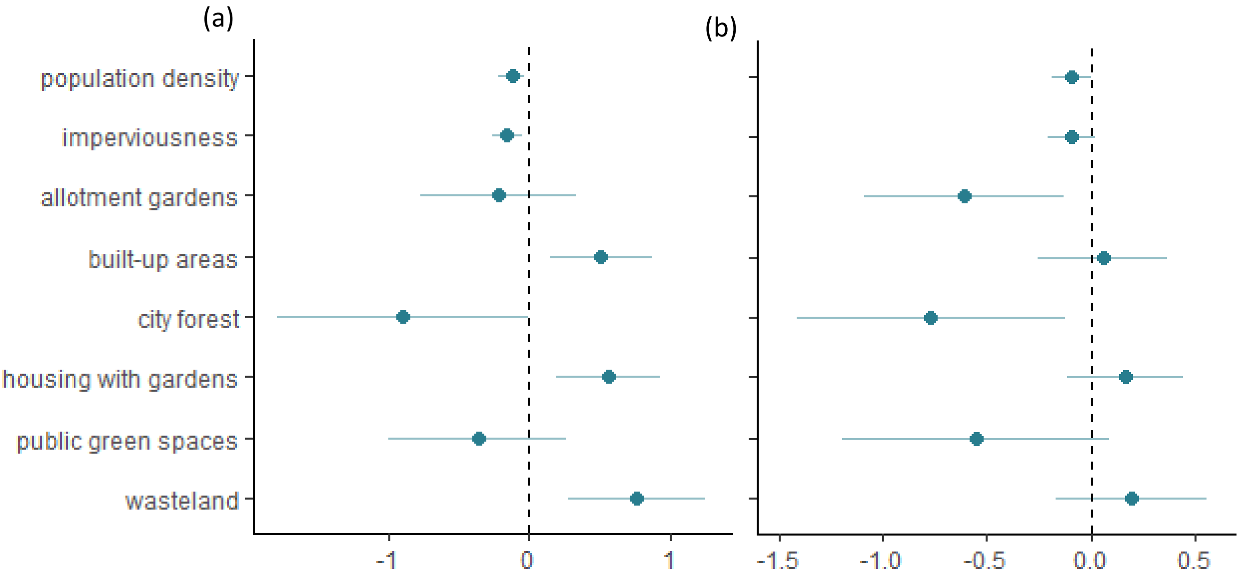

Figure 5: Selection probability of different covariates using all GPS data (a) and movement data only (b). Positive values indicate preference, negative values avoidance of landscape types in relation to the reference category grey spaces (indicated by the dashed line).

2. 5 Discussion

Our study shows that habitat selection in urban red foxes is influenced by several factors, including habitat type, the level of human presence and activity time. Foxes changed land use as a function of human activity patterns, preferring habitat types difficult to access for people during the day and being less selective during the night when human activity decreased.

Inter-individual variability and model selection

It has been described many times that home range sizes of red foxes (and other carnivores) can vary by one or more orders of magnitude, with larger home range size in areas of low resource availability and smaller territories in high-resource areas such as cities and suburbs (e.g., Morellet et al. 2013). In Berlin, home range sizes also varied by more than one order of magnitude, from very small to larger home range sizes (Table S1). These varying home range sizes as well as the variance detected in the model predictions on (movement) habitat selection revealed large scale inter-individual differences that were also apparent with a classic electivity index analysis by Fiderer et al. (2019) in a rural fox population. Larger than expected variation in life-history traits among individuals within a population apart from well-described trait-related differences such as sex (Fredga et al. 1994) or age (Charlesworth 1994) as well as contrasting life histories have been described for wildlife populations before (e.g. Van Noordwijk & De Jong 1986, Gaillard et al 2010). However, this insight is often not considered in ecological data analysis. We therefore selected a model that not only corrected for individual spatial and temporal dependence but also for inter-individual variability to generate robust models, even though classic resource selection functions and other comparable approaches produced more significant results. The inter-individual variability also highlights that sample sizes are crucial to investigate habitat use in a flexible predator.

Urban space use

The hypothesis that foxes do not select specific habitat types can clearly be rejected by this study. Overall, foxes preferred wasteland, residential houses with gardens and built-up areas and avoided city forests, public green spaces and allotment gardens. The strong avoidance of forested areas is in contrast to previous findings of a general preference for woodlands by foxes in rural areas (Fiderer et al. 2019). One explanation which is consistent with our other results that foxes avoid high human presence and population densities, may be the presence and activities of people within these urban forests. For instance, according to the "Association of German Foresters", one of the largest urban forests that was partly incorporated in the home ranges of two study foxes, is visited by an estimated double-digit million number of people every year. In terms of landscape, forests are probably the most natural, "greenest" areas in the city, whereas other green areas which included trees, grassland and shrubs were provided by the 2,500 publicly owned and accessible city parks. These parks were also avoided by foxes, presumably for similar reasons, whereas wasteland (including railways) were highly preferred habitats. Also, foxes did neither avoid built-up areas (Fig. 3) nor did they avoid areas with a high percentage of impervious surface (Fig. 2), although the latter is avoided when human population density is controlled for (Fig. 5). Cavallini and Lovari (1994) found that within their home range, foxes strongly and consistently selected the habitat with the densest cover for resting and their requirement of dense cover has been repeatedly demonstrated (e.g. Robertson et al. 2000, White et al. 2006). Such cover is available on wasteland areas, in public parks, in residential housing gardens and also in allotment gardens. Nevertheless, allotment gardens were also avoided compared to built-up areas and wasteland. Consequently, the hypothesis that foxes primarily prefer green and avoid grey areas can be rejected too. Not all wasteland areas are actually green spaces, they also include brown areas, building remains and abandoned industrials areas, and the covariate used here further included the city's railway lines. The selection probabilities for the mentioned landscape types as well as the avoidance of high human population density therefore indicates an avoidance behaviour towards human presence and activity. Studies in London (Harris 1977), Toronto (Adkins and Stott 1998) and Switzerland (Weber & Meia 1996) showed that a lack of regular disturbance by humans is important for foxes when they select den sites. In Melbourne, avoidance of humans was a major precondition for selecting natal den sites (Marks et al. 2006). The avoidance of areas dominated by human activity in Berlin was not limited to den or resting sites but also applied to general habitat use and movement behaviour (see also below).

Diurnal patterns

The fox is usually described as a predominantly nocturnal or crepuscular animal (e.g. Maurel 1980, Cavallini & Lovari 1994, Díaz-Ruiz et al. 2016). According to Maurel (1980), the diurnal activity of foxes is limited to the period of returning to the main burrow area. In contrast, we found that around one fifth of active movement fixes occurred between sunrise and sunset and foxes with access to rather undisturbed areas used large areas within their home ranges during the day (Table S1, Fig. S2). A camera trap study on activity patterns also reported variation among individuals regarding the extent of diurnal activities (Díaz-Ruiz et al. 2016). Monterroso et al. (2014) described the red fox as «facultative nocturnal species" and stated that such species may exhibit substantial flexibility to locally adjust their foraging strategies. Díaz-Ruiz et al. (2016) found that red fox activity rhythms were determined by human presence where human disturbance was high and that diurnal activity decreased in areas with higher levels of human disturbance whereas the temporal overlap with prey activity was on average low. Our results suggest that the selection of undisturbed habitat such as wasteland or of space with low human activities such as residential houses with gardens was more pronounced during times of human activity. Built-up areas were also preferred at all times, but this is difficult to interpret with respect to their level of human-related disturbance as they include a variety of different structures including more and less disturbed spaces. Their positive selection nevertheless indicates that foxes did not avoid houses or artificial structures *per se*. Allotments appeared to be more strongly avoided during human activity times, which seems logical as people do not reside in those gardens so they are mainly used during daytime. Finally, the avoidance of increasing human population density seems more distinct at times of human activity (Fig. 3 c-d).

Vigilance behaviour

As stated above, Cavallini and Lovari (1994) found that foxes preferred habitats with dense cover for resting. They also described this finding as unexpected, as in their study area foxes were not hunted for about ten years. They assumed that either more favourable thermic conditions or "the permanence of a previously adaptive behaviour (with either a genetic or a cultural basis) may explain this tendency" (p 245). Our study is consistent with the latter explanation and suggests that foxes may have maintained a certain level of shyness or vigilance towards humans as a measure of predator avoidance behaviour even after dozens of "hunt-free" urban fox generations. This is in line with previous findings regarding human-driven dispersal patterns (Kimmig et al. 2019) based on a transfer of the "landscape of fear" concept from feeding ecology (Laundré et al., 2010). Railway lines were included in the wasteland category as both elements share their inaccessibility to people and were present in all fox home ranges. When available, foxes selected these areas with one fox almost entirely living (and dying) on a railway segment. Considering the mortality risks associated with this urban infrastructure, the pressure to find spaces free of people might be high.

Movement

The overall pattern of habitat use was similar for the full dataset and the movement only dataset, but selection preferences were less distinct for movement data (except for forest and allotment gardens). This is in line with our finding that selective patterns are less distinct at night-time (when human activity is reduced), when most fox movements occur. The habitat use during active movement nevertheless showed the described preference patterns.

Mammalian carnivores mainly move to find and capture food, avoid competitors, avoid predators, find mates, and scent-mark and otherwise communicate with conspecifics (Powell 2012) but their movement is restricted by humans (Tucker et al. 2018). Although food availability was not included in our study, the avoidance of feeding competitors as a driving factor for habitat selection seems less important in urban areas where food resources are ubiquitously available and abundant (Macdonald 1983, Macdonald & Johnson 2015). Accordingly, previous studies showed group-living and less territorial behaviour in urban red foxes and a territoriality which is generally not too strict (Baker et al. 1998, Baker et al. 2004, Cavallini 1996). Our results suggest that the avoidance of predators in terms of humans, drives fox habitat use during movement. Interestingly, our results also showed that the avoidance of allotment gardens is more pronounced when focusing on fixes during active movements, although these areas probably provide the largest amount of food besides built-up areas. Because of their mixed plant composition, allotment gardens provide insects, earthworms, rodents, bird nests and voles as well as crops, fruits and leftovers from humans. Built-up areas were also less selected during fox activity. This could indicate that they are also important for resting behaviour, which seems likely in cases when wasteland is not always available to an individual fox and large city areas consist of apartment blocks without residential houses, making backyards and verges along apartment blocks the only protected patches with cover.

More fine scale analysis of movement data in combination with high resolution geographic data which reveal information on local food availability may help to further clarify the role of resource distribution on space use and movement patters. We conclude that human presence is a main driver of space use and activity patterns in urban areas in a commensal species.

2.6 Acknowledgements

We are indebted to the Stiftung Naturschutz and the Leibniz Institute for Zoo & Wildlife Research for providing funding for this study. We further acknowledge Konstantin Börner, Janina Radwainski and Frank Goeritz for their contribution to field work. Finally, we would like to thank all authors for their contributions to this manuscript.

2.7 Author contributions

H.H., M.B., S.E.K. and S.K-S. designed this research. K.B. and S.E.K. carried out field work. M.W. and S.E.K. generated the geo files and conducted data preparation. A.P. and S.E.K. analysed the data. S.E.K. wrote the manuscript. All authors contributed with substantial revisions to the manuscript.

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Supplemental information for: The limits of serenity - avoidance behaviour towards humans in an opportunistic predator

Content

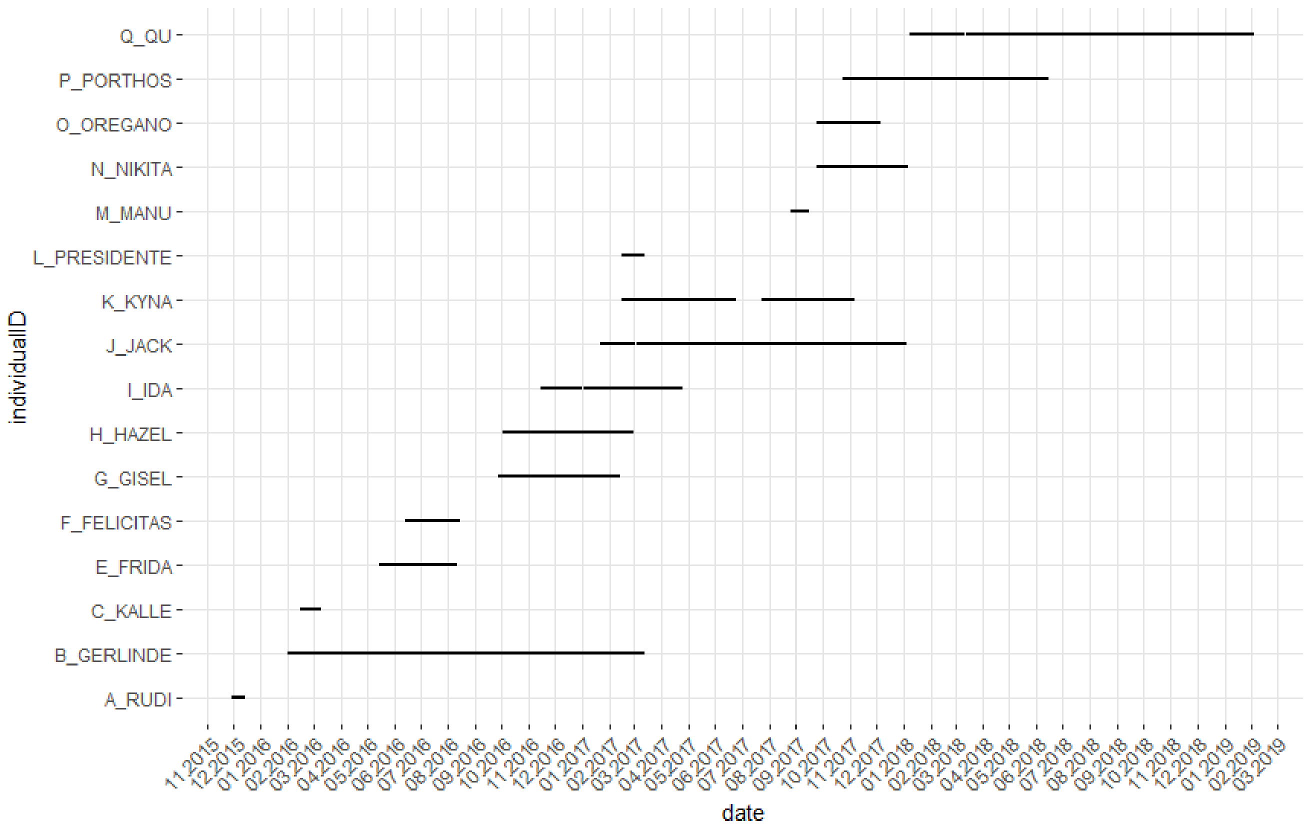

Figure S1: Deployment times of the 16 radio-collared study foxes.

Table S1: Summary on age, sex, weight, deployment and home range sizes of the 16 study animals. Due to short deployment times and high fluctuations, no proper home range estimation was possible for individuals marked with * symbol. HR = home range.

Animal ID	**sex**	**age [years]**	**weight [kg]**	**duration [days]**	**HR 95% kernel [ha]**	**HR [ha] day**	**HR [ha] night**	**HR [ha] dawn**	**HR [ha] dusk**
Rudi	m	1	6,1	13	*				
Gerlinde	f	1,5	5,3	403	100	67	107	78	84
Kalle	m	1,5	6,05	21	220	32	248	41	47
Frida	f	1,5	4,8	84	176	50	242	182	88
Felicitas	f	1,5	4,86	59	122	41	156	125	60
Gisel	f	1,5	4,9	159	151	117	151	112	114
Hazel	f	4,5	5,8	145	44	43	44	51	38
Ida	f	1,5	4,8	160	151	61	154	75	89
Jack	f	3	7,1	344	80	48	89	94	47
Kyna	m	1,5	5,6	263	72	34	75	37	45
Presidente	f	7	6	23	95	11	95	61	20
Manu	m	2	7	17	*				
Nikita	m	1,5	6,8	102	31	5	32	10	2
Oregano	f	6	5,7	71	14	2	15	6	5
Porthos	m	1,5	6,1	240	143	57	160	89	69
Qu	m	1,5	5,9	389	43	7	48	25	21

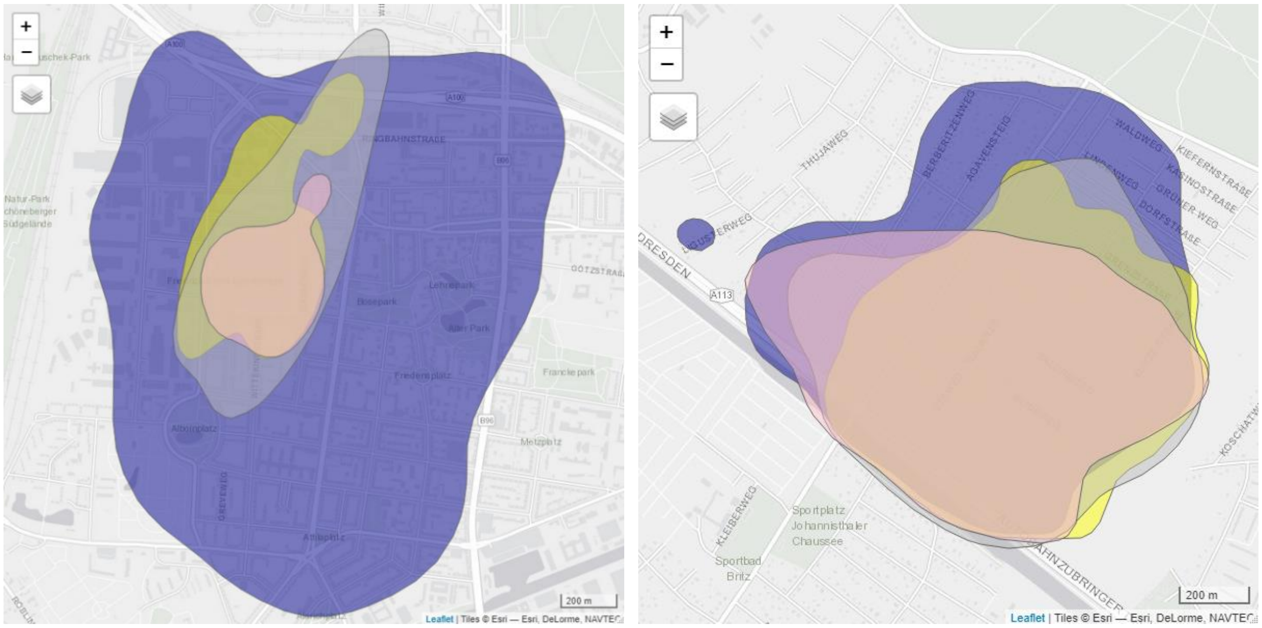

Figure S2: Example of diurnal home range visualizations for two different study foxes. On the left, fox “Kalle”, living mainly in built-up, crowded areas, on the right, fox “Gerlinde”, living mainly on undisturbed wasteland. Blue colour indicates the kernel density estimate based on nocturnal points, yellow the estimate for points measured during daytime, pink during morning twilight and grey during evening twilight.

Table S2: GlmmTMB output for the land use raster (signifiance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

Covariate	Estimate	Std. Error	z value	Pr(>|z|)	significance
Public green spaces	- 0.4216	0.3113	-1.354	0.1757	
City forest	-0.879	0.4464	-1.970	0.0488	*
Wasteland	0.5427	0.2711	2.002	0.0453	*
Built-up areas	0.1899	0.1530	1.241	0.2146	
Housing with gardens	0.2506	0.1602	1.564	0.1177	
Allotment gardens	-0.3077	0.2571	-1.197	0.2313	

Table S3: GlmmTMB output for the land use raster based on GPS data taken during daytime (signifiance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

Covariate	Estimate	Std. Error	z value	Pr(>|z|)	significance
Public green spaces	-0.2680	0.4032	-0.665	0.50632	
City forest	-0.9735	0.6004	-1.621	0.10494	
Wasteland	1.1711	0.4154	2.819	0.00481	**
Built-up areas	0.4520	0.2793	1.618	0.10562	
Housing with gardens	0.4783	0.4146	1.154	0.24865	
Allotment gardens	-1.8226	1.0267	-1.775	0.07588	.

Table S4: GlmmTMB output for the land use raster based on GPS data taken during night-time (signifiance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

Covariate	Estimate	Std. Error	z value	Pr(>|z|)	significance
Public green spaces	-0.42352	0.28749	-1.473	0.1407	
City forest	-0.76015	0.40554	-1.874	0.0609	.
Wasteland	0.31959	0.22207	1.439	0.1501	
Built-up areas	0.05272	0.13024	0.405	0.6856	
Housing with gardens	0.08445	0.08799	0.960	0.3372	
Allotment gardens	-0.31752	0.22788	-1.393	0.1635	

Table S5: GlmmTMB output for the full model based on the complete data set (signifiance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1).

Covariate	Estimate	Std. Error	z value	Pr(>|z|)	significance
Population density	-0.12056	0.04735	-2.546	0.01090	*
Imperviousness	-0.15277	0.05365	-2.847	0.00441	**
Public green spaces	-0.36298	0.32320	-1.123	0.26139	
City forest	-0.89933	0.45618	-1.971	0.04868	*
Wasteland	0.76608	0.24993	3.065	0.00218	**
Built-up areas	0.50749	0.18358	2.764	0.00570	**
Housing with gardens	0.55870	0.18585	3.006	0.00265	**
Allotment gardens	-0.21658	0.27880	-0.777	0.43727	

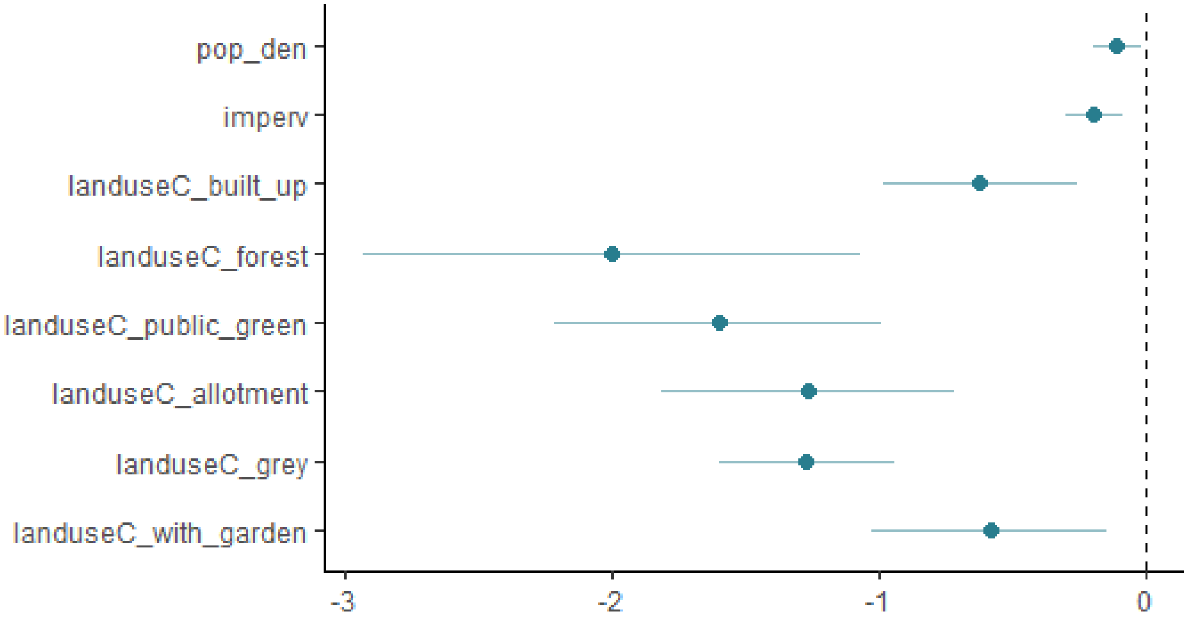

Figure S3: Full model for the complete dataset with wasteland as a reference value.

Table S6: GlmmTMB output for the full model with wasteland as the reference covariate (significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1).

Covariate	Estimate	Std. Error	z value	Pr(>|z|)	significance
Population density	-0.10897	0.04691	-2.323	0.020174	*
Imperviousness	-0.19668	0.05476	-3.591	0.000329	***
Public green spaces	-1.60300	0.31042	-5.164	2.42e-07	***
City forest	-2.00287	0.47437	-4.222	2.42e-05	***
Grey spaces	-1.27250	0.16766	-7.590	3.21e-14	***
Built-up areas	-0.62368	0.18501	-3.371	0.000749	***
Housing with gardens	-0.58407	0.22563	-2.589	0.009636	**
Allotment gardens	-1.26848	0.27915	-4.544	5.52e-06	***

Table S7: GlmmTMB output for the full model based on the movement data subset (significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1).

Covariate	Estimate	Std. Error	z value	Pr(>|z|)	significance
Population density	-0.09118	0.04857	-1.877	0.0605	.
Imperviousness	-0.09190	0.05949	-1.545	0.1224	
Public green spaces	-0.55293	0.32763	-1.688	0.0915	.
City forest	-0.76810	0.32929	-2.333	0.0197	*
Wasteland	0.19722	0.18435	1.070	0.2847	
Built-up areas	0.05835	0.15989	0.365	0.7152	
Housing with gardens	0.16670	0.14014	1.190	0.2342	
Allotment gardens	-0.60597	0.24358	-2.488	0.0129	*

ADDITIONAL WORKS

Chapter 3: Inference of animal behaviour from acceleration data

Machine learning goes wild: Using data from captive individuals to infer wildlife behaviours

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Author contribution

The original idea to study acceleration data using red fox radio tagging was developed by Anne Berger (AB). The detailed concept was developed by Sophia E. Kimmig (SEK) and Wanja Rast (WR). Organisation and implementation of field work (trapping, radio-collaring and tracking) was done by SEK for all wild red foxes. Observation of the two captive red foxes was done by Lisa Giese (LG) under supervision of SEK. WR conducted data analysis, SEK and WR developed data visualisation. SEK and WR wrote the manuscript. AB contributed with substantial revisions to the manuscript.

RESEARCH ARTICLE

Machine learning goes wild: Using data from captive individuals to infer wildlife behaviours

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Abstract

1. Remotely tracking distinct behaviours of animals using acceleration data and machine learning has been carried out successfully in several species in captive settings. In order to study the ecology of animals in natural habitats, such behaviour classification models need to be transferred to wild individuals. However, at present, the development of those models usually requires direct observation of the target animals. 2. The goal of this study was to infer the behaviour of wild, free-roaming animals from acceleration data by training behaviour classification models on captive individuals, without the necessity to observe their wild conspecifics. We further sought to develop methods to validate the credibility of the resulting behaviour extrapolations. 3. We trained two machine learning algorithms proposed by the literature, Random Forest (RF) and Support Vector Machine (SVM), on data from captive red foxes (*Vulpes vulpes*) and later applied them to data from wild foxes. We also tested a new advance for behaviour classification, by applying a moving window to an Artificial Neural Network (ANN). Finally, we investigated four strategies to validate our classification output. 4. While all three machine learning algorithms performed well under training conditions (Kappa values: RF (0.82), SVM (0.78), ANN (0.85)), the established methods, RF and SVM, failed in classifying distinct behaviours when transferred from captive to wild foxes. Behaviour classification with the ANN and a moving window, in contrast, inferred distinct behaviours and showed consistent results for most individuals. 5. Our approach is a substantial improvement over the methods previously proposed in the literature as it generated plausible results for wild fox behaviour. We were able to infer the behaviour of wild animals that have never been observed in the wild and to further illustrate the credibility of the output. This framework is not restricted to foxes but can be applied to infer the behaviour of many other species and thus empowers new advances in behavioural ecology.

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Data Availability Statement: The data of the captive foxes from which the machine learning model was built is available on Dryad: DOI https://doi.org/10.5061/dryad.gtht76hh. The designed R codes are available on GitHub: https://github.com/wanjarast/accelerateR. The data of the wild foxes is stored on Movebank and can be shared upon request.

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Introduction

Animal-borne sensors such as temperature loggers, salinity loggers or microphones are used to study a wide variety of parameters in wild animals without disturbance by human

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observers [1]. In the study of movement ecology of species [2], animal-borne sensors make it possible to track the locations of wild animals. The first attempts to remotely track animal locations were made in the 1960s through VHF telemetry [3]. In more recent years it has become common practice to track animal locations with satellite systems [4], enabling researchers to study where individuals dwell. However, the spectrum of ecological questions that can be addressed by using location data alone is limited. By combining such data with behavioural data, more in-depth studies of species will become possible [5]. Yet, in contrast to recording locations, remotely tracking the behaviour of free-ranging animals is not well established at this point.

The principal underlying remote-tracking of behaviour is to attach accelerometers to animals to record their body movement. The first major study utilizing acceleration data to study the behaviour of animals was conducted in 1996 [6]. Since then, many studies have shown that acceleration data can be used to infer the behaviour of animals by employing various machine learning algorithms [5, 7]. To train these algorithms for pattern recognition and data classification, the acquisition of acceleration data was coupled with direct observation of the behaviours of the tagged animals. Using one portion of this ground-truthed data set to train the algorithm and another portion to infer behaviour from it allows validation of the inferred behaviour.

Extrapolating behaviours from acceleration data of wild individuals is a challenge since it is often impossible to test whether the extrapolated behaviours are correct or not. Some models were trained and validated on the same wild individuals [8–10], which requires direct observation of the studied individuals at least for a certain period of time. However, the promising advance of behaviour classification through machine learning is the ability to study the behaviour of wild animals without observing (and possibly disturbing) them. Furthermore, direct observation may often not be a feasible option, especially when target species are elusive or cryptic.

For other models, additional sensors such as GPS [11] or depth and speed sensors for aquatic species [9, 10, 12] were employed to identify the behaviours executed. In these cases, the information from the additional sensors was used to investigate the behavioural context the animal was in at the time of data recording, in order to delimit likely behaviours. For studies in which no validation was possible, various behaviours were grouped into broad, easily distinguishable categories to reduce confusion of similar behaviours [13, 14]. Thus, accurately inferring distinct behaviours of wild individuals still poses a problem.

The Random Forest (RF) and the Support Vector Machine (SVM) are popular approaches to infer animal behaviour from acceleration data and have yielded good results under training conditions [5, 15]. Yet, to our knowledge, there are no studies successfully transferring a behaviour classification model trained on captive individuals to wild individuals.

To study the complex behaviour- or movement ecology of wild animals, however, a valid data set of linked GPS locations and behavioural data is needed. In this study, we, therefore, aim to test the capacity of different machine learning algorithms in inferring the behaviour of wild foxes (*Vulpes vulpes*) from acceleration data. We provide a framework to infer the behaviour of wild red foxes based on an Artificial Neural Network (ANN) trained on captive red foxes.

Our framework further addresses the issues of working with small training data sets (a common obstacle in wildlife- and conservation research) by using a new approach to efficiently exploit the given data set. Finally, we suggest how to validate the inferred behaviours when observation of free-ranging individuals is not a feasible option. We propose four strategies to assess the credibility of the output by combining the classified behaviour with GPS and temporal information. The study set-up, together with our novel approach, enables us to test the use of machine learning for behaviour classification and to empower behaviour classification of wildlife through acceleration data in the future.

Material and methods

Data collection and acceleration logger setup

Animal catching and handling have been approved from the State Office for Health and Social Affairs, department of veterinary affairs (permit number: IC113-G0211/15) and the ethics committee of the Leibniz Institute for Zoo and Wildlife Research in Berlin (permit number: 2015-03-04) and have been conducted according to applicable national and international guidelines. Approvals have been received prior to beginning research. To reduce stress during handling, all foxes got anesthetized before the deployment of radio collars. For anaesthesia we first used a long established mixture of Xylazin (10-16mg/kg) and Ketamin (12-20mg/kg) and later switched to an improved mixture of Ketamin (4mg/kg), Medetomidin (70μg/kg) and Midazolam (0,6mg/kg) that is better tolerated.

For gathering the acceleration and GPS data sets, used in this study, we deployed UHF-GPS collars (“1C-light” and “1C-heavy”, E-obs GmbH, Munich, Germany; Fig 1) on adult red foxes, both, in captivity and in the wild. Both captive and wild individuals were tagged with the same type of sensors and acceleration data logger settings. Captive individuals were observed to train and test the models and wild individuals were used to apply them. For the training data set, two individuals (female, approx. 8 years old) were collared in a game park enclosure in the north-west of Berlin between November 2015 and June 2016. Their enclosure mainly consisted of a sandy and stony substrate and was partially covered with concrete, grass and weeds. Several trees, tree roots, piles of stones and cement tubes provided a heterogeneous environment with both opportunities to hide and climb. One of the cement tubes led to an artificial, observable den. The two foxes were chosen to be collared, because of their lacking fear towards visitors and noises and the resulting possibility to be observed outside their den for several hours per day. For the field dataset, data from wild foxes were used that were radio-collared by Kimmig et al. in the city of Berlin, Germany, between 2015 and 2018. In total 17 wild individuals (10 female, 7 male) were caught. Out of those, for 9 individuals (7 females, 2 males), three consecutive months of data were available and they were therefore included in the analysis. All individuals were adults (with ages ranging from 1.5 to 7 years) and their urban and suburban habitats were characterized by a heterogeneous structure, including green spaces as well as concrete.

The acceleration loggers that were embedded in the UHF-GPS collars were set up to measure acceleration in short intervals at a frequency of two minutes. Data was recorded for three axes perpendicular to each other at a sampling rate of 33.33Hz per axis. There were 110 acceleration measurements taken for each axis in each measurement interval. Resulting from the sampling rate and the number of measurements for each axis the duration of each recording interval was 3.3 seconds. We refer to a single recording interval as a burst.

To train the algorithms we used the raw ground-truthed data of the captive foxes that were observed during the recording of acceleration data. A specific UHF-pinger signal indicated the start of each burst for the observer who then noted the displayed behaviour. All measured behaviours had been previously classified in an ethogram that was established through observations before and after collaring the individuals (with all steps conducted by the same observer). It contained the following behaviours: feeding, grooming, resting, caching, trotting and walking (for a detailed description of the behaviours see S1 Table). The pinger signal could be detected acoustically with a UHF Wide Range Receiver that was set to the unique frequency of the collars (see [16]) and was not audible to the foxes.

During a burst, the animal in focus was observed closely and the behaviour was noted. Each observation was linked to the corresponding acceleration burst via the unique timestamp.

Fig 1. Camera trap picture of a wild red fox ("Gerlinde"), collared in Berlin in 2016. The arrows symbolize the X-, Y- and Z-axis (corresponding to sway-, surge- and heave-motion).

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Due to slight shifts in the collars' timestamps, the raw acceleration data of a number of consecutive bursts—ideally encompassing a distinctive change in behaviour (e.g. resting followed by trotting)—was visually inspected and compared to the noted behaviours. The timestamps of observations were corrected accordingly.

After excluding all bursts containing more than one behaviour, 4159 bursts of six different behaviour classes were used as the input for the model training (feeding: 367, grooming: 1140, resting: 2114, caching (bury food to consume it later): 197, trotting: 179, walking: 162).

Data preparation

We calculated summary statistics from the raw acceleration data, separately for each burst, to serve as predictors for the machine learning algorithms. The following predictors were calculated per axis: mean, standard deviation, inverse coefficient of variation, variance, skewness and kurtosis. Additional predictors represent combinations of all three axes and were calculated according to the corresponding literature: q [5], pitch and roll [17] and overall dynamic body acceleration (ODBA) [18]. In addition to the summary statistics, we added the whole spectrum of a Fast Fourier Transformation of each axis to the set of predictors. As most of the time-related information in the raw acceleration data is lost when calculating the summary statistics we decided to use the full spectrum to utilize this information. For a complete list of predictors see S2 Table. We performed all data transformations and the construction of the ANN in R [19] and Rstudio [20]. The sum_data function in the accelerateR package (W. Rast, unpublished data.) was used for summary statistics and Fast Fourier Transformation calculation.

Data classification

Established methods: Support vector machines (SVM) and random forest (RF). Support Vector Machines separate data of different classes from each other by constructing a hyperplane between them. Classification of new data is subsequently based on their relative position to the hyperplane. By default, the classification is binary. For applications with multiple classes, more hyper-planes between classes will be constructed [21]. We used the implementation of an SVM in the R package "e1071" [22] with the kernel type "radial".

Random Forests are an improvement of the classical Classification and Regression Trees (CART) [23]. While in CART all predictors are used, the RF picks a random subset of predictors to fit a tree. This is repeated several times, and the final prediction is the result of all trees combined by a majority rule [24]. We used the implementation of an RF in the R package "randomForest" [25] with the standard settings using 500 trees.

Artificial neural network (ANN). ANNs are similar to biological neural networks and consist of multiple nodes that are distributed over several layers and interconnected [26]. Nodes are activated based on the input variables (predictors) and an activation function. In the simplest cases, this function is a summation of all input variables that are passed to a specific node. These functions also include weights that change the influence of every input variable and are set during the training phase. For training, a ground-truthed data set is needed on which the ANN establishes the node connections and the weights so that the output of the ANN corresponds to the target classes of the model data. The activation or non-activation of nodes serve as input for the next layer of nodes. The last layer usually consists of nodes representing the target classes. Their activation leads to the assignment of data to a class.

For our study, we chose a three-layer network with the output of the last layer being a specific behaviour class. We used a feed-forward type architecture for the ANN and used the Keras package [27] to implement it.

Moving window

One strategy that has been tested with continuously recorded data is to apply a moving window to partition the acceleration data and to compute summary statistics for each of the resulting segments. In different studies, these windows could partially overlap or not overlap at all [28–30]. An application example very similar to our approach is the assessment of car driver aggressiveness using continuous data by Ferreira et al. [31]. However, to our knowledge, this approach has never been used on burst data in wildlife ecology.

We applied a moving window to every recorded burst to increase the sample size of our data set since it was found that ANNs show better performance with increasing sample size [32, 33] and require large training data sets [34]. In the first set, this window reduced the amount of data within the burst from the original 110 measurements down to a subset of the window length. We then computed the summary statistics and Fast Fourier Transformation (S2 Table) for this subset. In a second step, the window was moved by one position so that the first measurement of every axis was removed and one new measurement for every axis was added to the end of the window (see Fig 2). We then computed all variables for the second

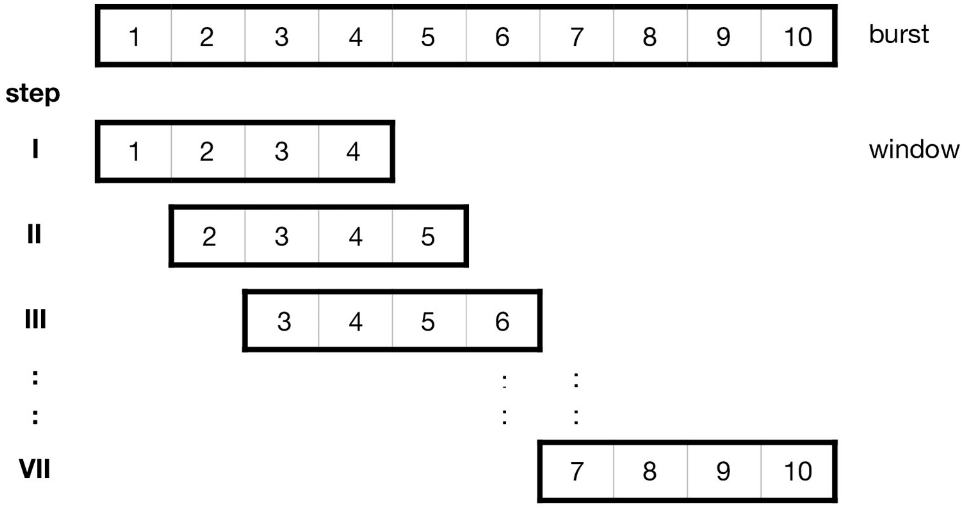

Fig 2. Schematic representation of the moving window approach: Starting at the beginning of a data set ("burst", here n = 10), a fixed number of consecutive data ("window", here n = 4) is taken out and analysed. In the further step-by-step analysis, the window is shifted by one data set until the window has reached the end of the complete data set (7 steps in the schematic example).

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window and so forth. The window was moved until it included the last measurement of the original burst, resulting in a number of predictor sets representing the same burst. In contrast to extracting random subsets, this approach preserves the order of the data for a specific behaviour, and we were able to calculate the Fourier spectrum which is dependent on the correct order of measurements.

Model evaluation

To evaluate the model performance of all three machine learning algorithms, we trained them on 70% of the data (training data). We then inferred the behaviour for the remaining 30% (test data) by classifying them with the trained model and assigning a specific behaviour to each burst accordingly (or assigning "other", respectively as described below). Since the number of observations per behaviour class differed, we split the data of each behaviour class separately in a random fashion so that the original proportions of behaviour counts were similar in the training and test data sets. We applied the moving window to the training and test sets after the split. We calculated the recall (true positives / (true positives + false negatives)) and precision (true positives / (true positives + false positives)) [7] for each behaviour. For the sake of completeness, we also calculated the accuracy ((true positives + true negatives) / total number of samples). For comparison with other models we calculated Cohens Kappa with $Kappa = (p_o - p_e)/(1 - p_e)$ with $p_o = \sum_1^c (TP_c/n)$ and $p_e = \sum_1^c ((TP_c + TN_c)/n * (TP_c + FP_c)/n)$. With <n> being the total sample size and <c> the number of classes [35].

To reduce confusion of behaviours, a threshold was set for the ANN assignments. Only behaviour assignments that exceeded a probability of 0.7 were accepted. All assignments below that threshold were classified as "other" behaviour. This was necessary to account for the fact that captive individuals may not execute the full range of behaviours available to the species, which would lead to some behaviour (e.g. hunting or fighting) not being included in the model. If wild individuals displayed any of these behaviours, they could be incorrectly classified as one of the behaviours included in the model. We expect that such classifications would be assigned at low probability so that we can avoid these errors by implementing the threshold. Similarly, recordings in which the individual changed its behaviour during a burst should not be characteristic for any specific behaviour and therefore should also fall below the threshold.

Model selection

Artificial Neural Networks are used for a variety of tasks such as image recognition, sentiment analysis or regression. The necessary sample size and ANN architecture depend on the specific task [36]. Finding the optimal properties for the best performing ANN is not achieved by a scientific method but rather by trial and error [33]. To find the best window size we trained the ANN on window sizes from 20 to the full 110 and finally decided on 79. We evaluated all models by calculating the recall, precision and the proportion of "other" behaviours. As recall and precision are calculated for each behaviour, we first computed their means and then calculated the mean of the resulting mean recall and precision as well as the proportion of "other" behaviours. The latter was subtracted from one to be on the same scale as recall and precision. A General Additive Model (GAM) was applied to the calculated means for all window sizes. We calculated the slope m of the GAM fit for each window size using the difference quotient $m = (\Delta y_n - \Delta y_{n-1})/(\Delta x_n - \Delta x_{n-1})$. Variable x corresponds to the window size and y to the calculated model performance, n corresponds to a specific window size and n-1 to the previous window size. A window size of 79 provided the best trade-off between small window size and high performance (see "Model selection" in the Results).

Application to wild individuals

For our subsequent analysis of behaviour inference, we selected wild foxes for which at least three consecutive months of acceleration data were available (N = 9). We considered all months in which data was recorded for at least half of the month. In addition to the acceleration data, the tags recorded GPS locations every four minutes for the first eight weeks, after that every 20 minutes (GPS for fox "Gerlinde" was only recorded every 20 minutes). Using acceleration informed GPS measurement, this interval was reduced to every four hours when a fox was inactive. We trained all three classification models on the complete ground-truthed dataset of the captive foxes and applied the trained model to classify the data of the wild foxes. As the moving window results in multiple behaviour outputs for each burst, only one behaviour was assigned to each burst, following majority rule. We consider all classifications within one burst as equal and determine the absolute majority.

Validation of behaviour assignments

We assessed the plausibility of the ANNs' behavioural assignments by examining the following four aspects: (i) biological credibility of the behaviour assignments (ii) consistency over individuals and time (iii) coherence with the GPS data and (iv) coherence with ODBA.

To address biological credibility (i), we calculated the time-dependent composition of behaviours throughout the day and compared it to the literature on fox behaviour. As seasonal shifts can influence behavioural compositions, we separated the behaviour assignments by month. For each day within a single month, we counted the number of assignments of each behaviour (for each minute covered by the tag schedule) in the 24 hours. We further used the corresponding plots to (ii) visually compare the daily patterns over time and between individuals. (iii) We incorporated the given GPS information of the free-ranging individuals because we expected the GPS data to correspond with specific behavioural classes. For instance, spatial clustering of GPS data should correspond with stationary resting behaviour. We treated points as a cluster when consecutive GPS points were within a 50m radius of the first GPS point of that cluster. Points recorded more than 50m away were defined as the first point of a new cluster. Since it was possible that clusters consisted of only a single point, we only considered behaviour assignments to be spatially clustered when at least 10 classified behaviour items were assigned to the same cluster. We then calculated for each behaviour the proportion of behaviour assignments that were within a cluster. In addition, we investigated the coherence of GPS based speed measure and movement-related behaviour classifications (trotting and walking). We, therefore, calculated the speed of the moving animal based on the spatial and temporal distances between consecutive GPS points. Due to independent schedules, GPS and acceleration data were not recorded exactly simultaneously. Hence acceleration data that was recorded within 10 seconds of a GPS measurement were considered. Finally, we (iv) compared the temporal distribution of ODBA values and behaviour assignments by constructing actograms using accelerateR.

Results

Training conditions: Captive foxes

We could classify all six behaviours during the validation using SVM and RF. Classification success differed between the behaviour classes for both algorithms. We achieved the best classification success for resting and the lowest for caching and walking (Table 1). The confusion matrices (S3 and S4 Tables) showed that grooming and walking were confused more often compared to other behaviours. Recall can be interpreted as the proportion of behaviour events that were correctly classified. Feeding (SVM), for example, had a recall of 0.43, meaning that 43% of all feeding events were correctly classified as feeding. Precision can be interpreted as the probability for an assignment to be correct. Feeding had a precision of 0.58, meaning that a single assignment of feeding is correct with a chance of 58%. Both algorithms show comparable results only for resting. The SVM performs worse for all other behaviours. Our initial testing showed that the SVM performed better without the addition of the FFT spectrum but we kept the model this way to ensure the comparability of all three models. We added the accuracy metric that is often used for model evaluation but will not endorse its use for this study: As accuracy uses the true negatives it is influenced by the large number of resting observations that we got. Since most of the resting data is classified correctly these data is treated as true

Table 1. Recall and precision of the classification output compared for support vector machine (SVM), random forest (RF) and artificial neural network (ANN). All algorithms are capable of classifying and inferring fox behaviour with a high success rate (exceptions are caching and walking for SVM and RF).

	Feeding	Grooming	Resting	Caching	Trotting	Walking
SVM						
recall	0.43	0.33	0.98	0.37	1.00	0.27
precision	0.58	0.70	0.98	0.21	0.19	0.36
accuracy	0.92	0.77	0.94	0.90	0.81	0.95
RF						
recall	0.70	0.93	0.92	0.68	0.96	0.43
precision	0.71	0.80	0.99	0.59	0.91	0.84
accuracy	0.94	0.91	0.95	0.96	0.99	0.98
ANN						
recall	0.83	0.88	0.96	0.67	0.96	0.74
precision	0.84	0.95	0.98	0.83	0.91	0.71
accuracy	0.93	0.92	0.95	0.95	0.96	0.95

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negatives for all other behaviour classes and thus resulting in higher accuracy values for those behaviour classes.

The Kappa values for the RF and SVM are 0.81 and 0.51 respectively. Our initial testing with training both models without the Fast Fourier Spectrum resulted in Kappa values of 0.81 and 0.78 for RF and SVM respectively. The performance of the RF remains the same while the performance of the SVM decreased due to the addition of the Fast Fourier spectrum.

Like RF and SVM, the ANN could predict all six behaviours during validation. Also, classification success differed between behaviour classes. The performance of the ANN is overall comparable to the RF but performs better than the SVM. The confusion of walking behaviour with grooming is reduced compared to the SVM and RF (S6 Table). The kappa value of 0.85 for the ANN was also higher than for the RF (0.81) and SVM (0.51). The proportion of assignments that did not surpass the threshold was 0.04.

Model performance of the ANN appears to be dependent on the window size (Fig 3) and decreases towards both ends of the window size spectrum. Smaller window sizes seem to have a stronger impact on model performance than larger window sizes. The GAM fit has its maximum at window size 79, with the slope of the GAM fit close to 0. We thus considered 79 to be the best trade-off between model performance and window size and used it for the final model (see Discussion).

Field conditions: Application to wild foxes

We here show the results for all wild foxes and plots for those two wild foxes ("Que" and "Gerlinde"), whose collars yielded data over a whole year. Graphic representation of all remaining individuals is presented in the supplemental material (S1–S7 Figs).

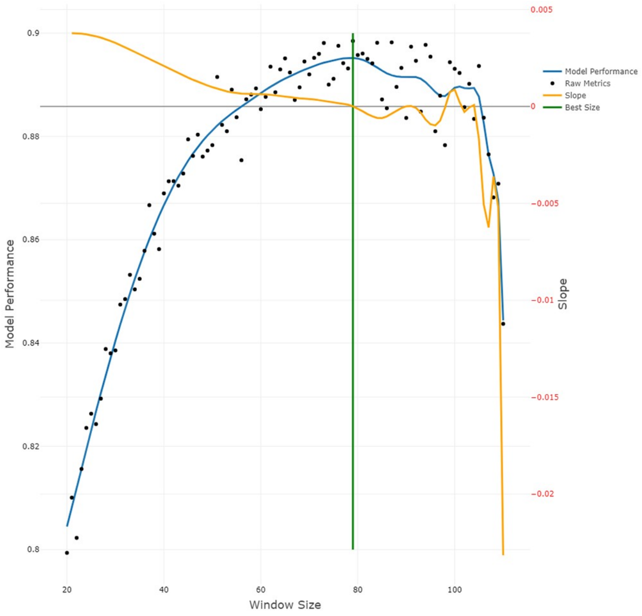

Fig 3. ANN model performance in relation to window size. Black dots show the computed performance values. The blue line is the result of a General Additive Model, k = 40 [37] fit. The y-axis on the left side labelled "Model Performance" corresponds to the Model Performance line (blue) and Raw Metrics points (black). The orange line is the calculated slope of the model performance, which corresponds to the y-axis on the right side labelled "Slope". The green vertical line represents the best window size of 79.

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When the trained SVM and RF models were applied to classify the behaviour of the nine wild foxes, all bursts were classified as grooming. No resting, caching, feeding, trotting or walking events were detected (Table 2). When applying the trained ANN to the wild fox data, all six behaviour categories were assigned in all nine individuals (Table 3). For the field data of "Que" and" Gerlinde", a proportion of 1% did not exceed the 70% threshold and was therefore labelled "other". For both foxes feeding, caching and walking were assigned at low rates.

Validation and credibility of the behaviour assignments

Biological credibility of the behaviour assignments (i) and consistency over individuals and time (ii). Looking at the time-dependent composition of each individual's behaviour (Fig 4, S1–S7 Figs), a similar pattern of behavioural composition over time is clearly noticeable (months without full data recording ought to be excluded for feasible interpretation). Clearly, there is a high proportion of resting behaviour during the middle of the day, while trotting is mostly inferred during dark hours. Trotting is also inferred more often than walking. There seems to be a seasonal change in resting behaviour, with resting events being more explicitly limited to the daytime in summer months. Feeding events are more often inferred during dark hours than during the daytime, when mostly resting and some grooming are classified.

In the comparison between individuals some differences emerge. Some individuals, e.g., show less trotting (S2 Fig), more walking (S3 Fig) or much more grooming than others (S7 Fig). Despite this variation, the general pattern of behaviour composition appears very similar across all individuals.

(iii) Coherence with GPS. Resting behaviour appears to be highly associated with GPS clusters (Fig 5A), while all other behaviours are inferred mostly outside of clusters. This also

Table 2. Number of occurrences of every classified behaviour for the wild foxes. Count of all behaviour assignments compared for support vector machine (SVM) and random forest (RF). Overall, all foxes show similar proportions of behaviours throughout their measurement periods. As all individuals were tagged for different time periods, the absolute number of assignments differs between individuals.

Individual	Measure	feeding	grooming	resting	caching	trotting	walking	other
SVM								
Gerlinde	count	0	289248	0	0	0	0	0
Gisel	count	0	102920	0	0	0	0	0
Hazel	count	0	103951	0	0	0	0	0
Ida	count	0	110337	0	0	0	0	0
Jack	count	0	243742	0	0	0	0	0
Kyna	count	0	159211	0	0	0	0	0
Nikita	count	0	72653	0	0	0	0	0
Porthos	count	0	149254	0	0	0	0	0
Que	count	0	274792	0	0	0	0	0
RF								
Gerlinde	count	0	289248	0	0	0	0	0
Gisel	count	0	102920	0	0	0	0	0
Hazel	count	0	103951	0	0	0	0	0
Ida	count	0	110337	0	0	0	0	0
Jack	count	0	243742	0	0	0	0	0
Kyna	count	0	159211	0	0	0	0	0
Nikita	count	0	72653	0	0	0	0	0
Porthos	count	0	149254	0	0	0	0	0
Que	count	0	274792	0	0	0	0	0

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Table 3. Number of occurrences of every classified behaviour for the wild foxes. Count and proportion of all behaviour assignments of the artificial neural network (ANN). Overall, all foxes show similar proportions of behaviours throughout their measurement periods. As all individuals were tagged for different time periods, the absolute number of assignments differs between individuals.

Individual	Measure	Feeding	Grooming	Resting	Caching	Trotting	Walking	Other
Gerlinde	count	2288	78194	171890	1016	30020	1664	4176
	proportion	0.005	0.23	0.61	0.007	0.12	0.02	0.01
Gisel	count	3019	16545	61405	1464	10766	7193	2528
	proportion	0.03	0.16	0.60	0.01	0.10	0.07	0.02
Hazel	count	2887	26014	57094	1319	8570	4874	3193
	proportion	0.03	0.25	0.55	0.01	0.08	0.04	0.03
Ida	count	1311	25162	65354	241	12848	1612	3789
	proportion	0.01	0.23	0.59	0.002	0.12	0.01	0.03
Jack	count	3420	136086	72507	523	17116	5092	8996
	proportion	0.01	0.56	0.30	0.002	0.07	0.02	0.04
Kyna	count	1070	45185	104491	266	6016	750	1433
	proportion	0.007	0.28	0.66	0.001	0.04	0.005	0.009
Nikita	count	2318	10430	47996	465	2678	7639	1127
	proportion	0.03	0.14	0.66	0.006	0.04	0.11	0.02
Porthos	count	1266	32624	91370	3555	16236	530	3673
	proportion	0.008	0.22	0.61	0.02	0.11	0.004	0.02
Que	count	1317	64092	166349	1875	32531	5749	2879
	proportion	0.008	0.27	0.59	0.004	0.10	0.006	0.01

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applies to all other wild foxes (S8A–S14A Figs). For the analysis of the correspondence between behaviours and GPS based speed measurements, we used only acceleration data recorded within 10 seconds of a GPS fix (for Que: 7%; Gerlinde: 5%). Resting events were classified at lower GPS-based speed than trotting events (Fig 5B, S8B–S14B Figs).

(iv) Coherence with ODBA. The actograms show that trotting is predominantly classified at times when ODBA values are high. Trotting, as well as high ODBA, occur mostly during night-time. Resting, in turn, is most often classified at times with low ODBA values (Fig 6). This is also valid for all remaining foxes (S15–S21 Figs).

Discussion

In the present study we sought to advance the abilities to remotely assess the behaviour of animals in the wild without directly observing (and respectively disturbing) the target animals. We therefore, tested the capacity of three machine learning algorithms (SVM, RF and ANN) to infer wild fox behaviour after training with a ground-truthed data set of two captive red foxes. The performances of the RF and the ANN were on similar levels under training conditions, the SVM overall performed worse than the other two. The ANN with the moving window approach, however, was able to infer caching and walking behaviour much better than the other two. Both RF and SVM generally performed well in inferring behaviour during validation (Table 1) and showed comparable results to other studies [5, 38, 39]. When applied to the wild foxes, however, they both failed to discriminate the different behaviours (Table 2).

The application of a model trained on one individual to a conspecific (that the model was not trained on), is crucial to bring this method into practical use, yet this remains a major obstacle. One rare example for a study classifying behaviours of conspecifics is the study

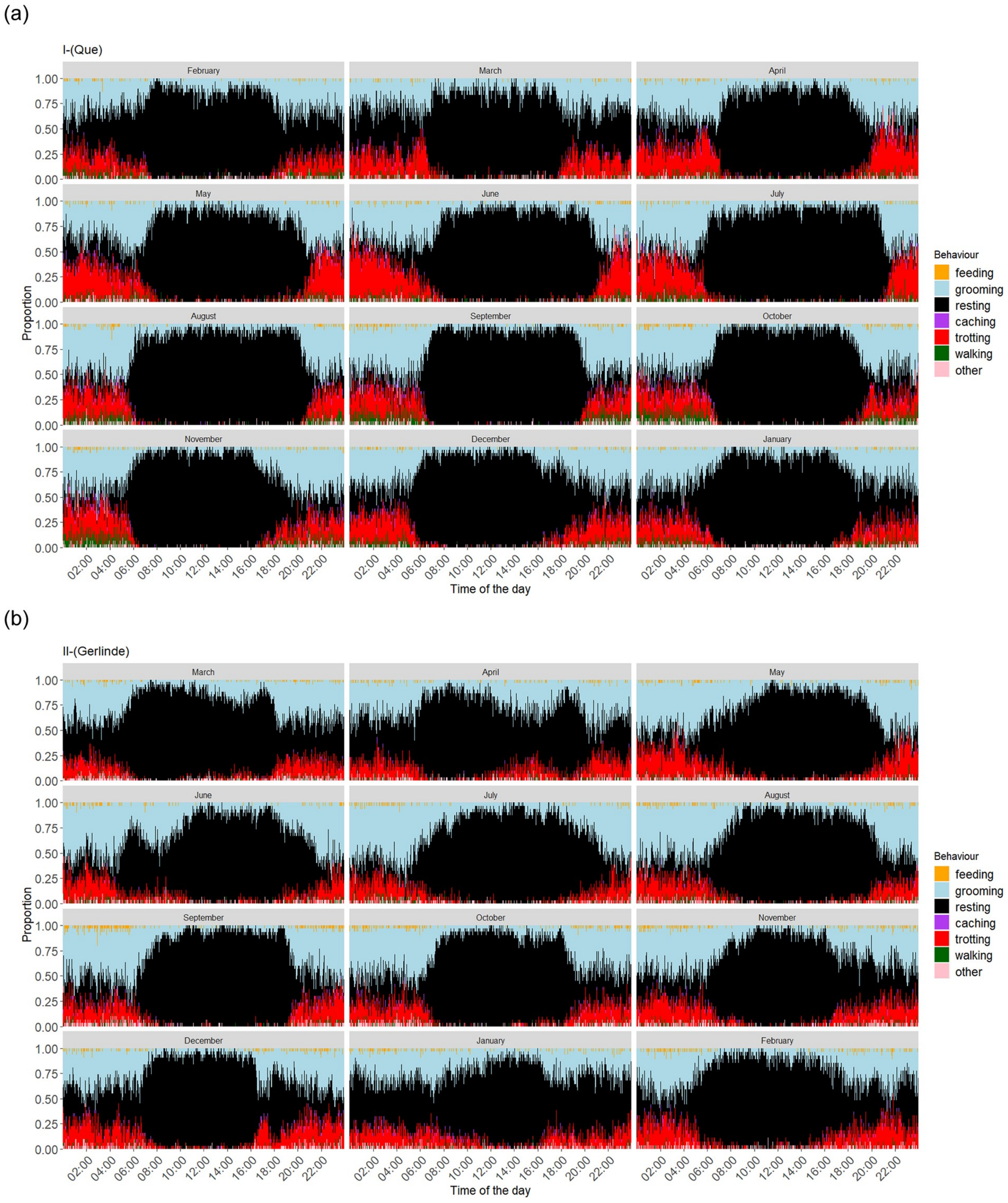

Fig 4. Time-dependent composition of behaviours of Que (I) and Gerlinde (II). Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data showed here span from February 2018 to January 2019 for Que and from March 2016 to February 2017 for Gerlinde.

https://doi.org/10.1371/journal.pone.0227317.g004

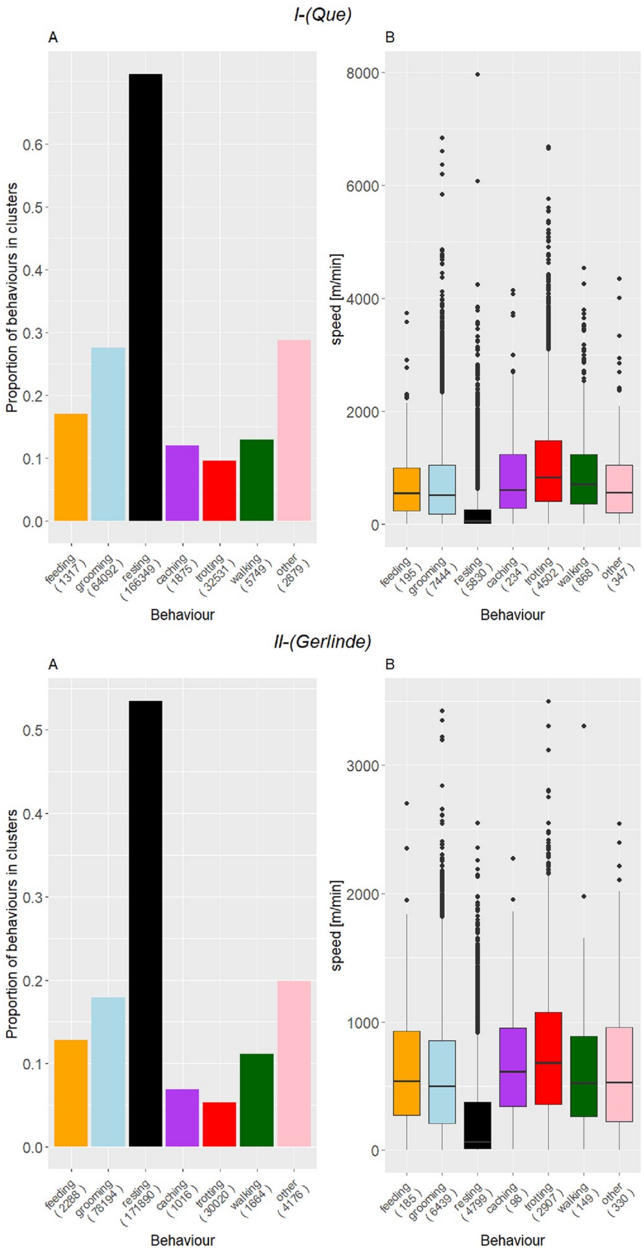

Fig 5. Behaviour assignments of Que (I) and Gerlinde (II) in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (I) Resting shows the highest association with GPS clusters (71%) and trotting the lowest (9%). Resting events are associated with significantly lower speed than trotting events (Wilcoxon rank sum test, W = 3024826, $p < 0.001$). (II) Resting shows the highest association with GPS clusters (53%) and trotting the lowest (5%). Resting events are associated with significantly lower speed than trotting events (Wilcoxon rank sum test, W = 2286090, $p < 0.001$).

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conducted by Moreau et al. [40] who used 3D-accelerometers on three goats to determine their head position (at accuracies of 61–82%) but could only predict three different traits, showing that inter-individual model projection leads to a reduction in the prediction's accuracy.

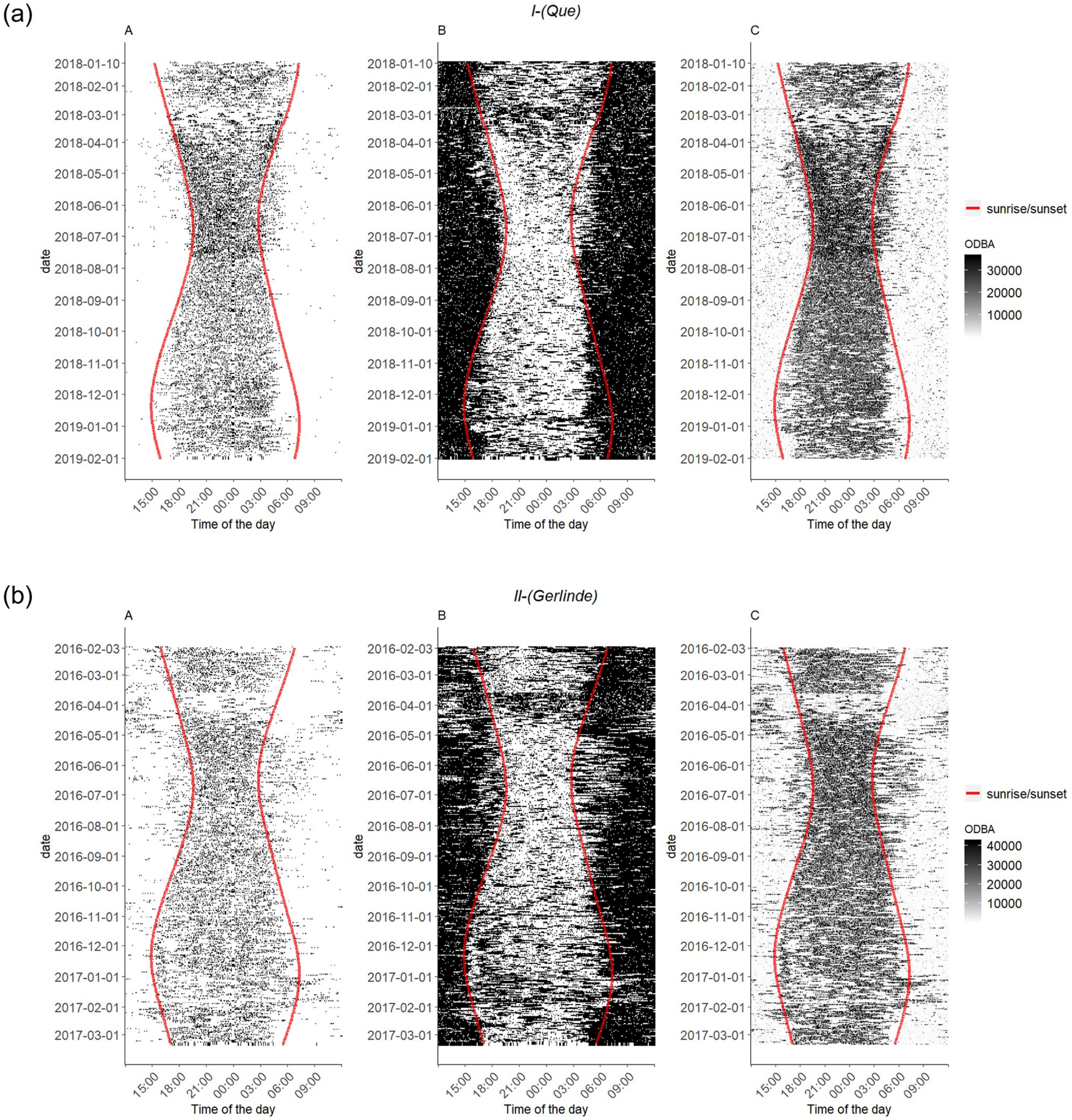

Fig 6. Temporal distribution of trotting (A), resting (B) and ODBA values (C) for Que (I) and Gerlinde (II). The red lines indicate sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was classified, whereas white spaces indicate the classification of all other behaviours. (B) Black spaces indicate times at which resting behaviour was classified, whereas white spaces indicate classifications of all other behaviours. (C) Higher ODBA values are indicated by darker spaces.

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Support Vector Machine (SVM) and random forest (RF)

When applied to the data from wild foxes, SVM and RF classified all behaviours as grooming. Considering that these datasets contain measurements for at least three months and that the GPS signal clearly showed the animals covering large distances, this classification is clearly unrealistic. In all cases, the models were trained and validated on measurements from the same captive individuals. Since both models correctly inferred behaviours of those captive individuals during validation, we suggest that SVM and RF thus failed to recognize similar patterns between wild and zoo-kept individuals because wild foxes showed characteristics in their behaviour that were too different from the captive foxes to be detected by those approaches.

This could be solved by training the model on ground-truthed data obtained from observing wild individuals that are logged [13]. However, as mentioned above, this is not always feasible, so it would be highly desirable to do trait classification without the necessity to directly observe wild animals. An additional problem of SVM and RF could be the recording of mixed behaviours. During model training, all recordings with mixed behaviours within one burst were excluded from the data set. However, it is a fair assumption that at least some recordings from wild individuals do contain more than one behaviour. Even though we implemented a probability threshold to account for these mixed bursts and—in the best case—classify them as "other" behaviour, these recordings might still pose an unclassifiable problem. Another issue is that grooming is classified to such a high proportion by both SVM and RF. As grooming behaviour is complex and may include different actions like licking, nibbling or scratching (see also S1 Table), the resulting behaviour class probably includes a broad range of characteristic summary statistics and may thus be more easily confused with other behaviour classes.

Lastly, we cannot consider natural behaviours that usually are not or very rarely observed in zoo-kept animals such as mating or territorial behaviour. These behaviours (as well as other, unknown behaviours) might very well have occurred in the wild where they could not have been classified because the algorithms were not trained on them. As possible for the mixed bursts, these untrained behaviours could be detected through the probability threshold and classified as “other”.

Another cause of the poor performance of the SVM and RF in predicting wild fox behaviour could be the small sample size of the data set of the captive foxes. With more observational data of the captive foxes, we might have been able to train a more robust model. An ideal model would thus be trained on more data. However, many studies in the field of wildlife ecology and conservation research face the problem of small sample sizes. Species may either be hard to observe or assess (e.g. due to remote or impassable habitats or an elusive nature of the species) or simply by cause of being too rarely distributed and/or barely zoo-kept. By introducing the moving window approach to wildlife behaviour recognition, we may actually have found a promising possibility to deal with these challenging conditions.

The artificial neural network (ANN)

The ANN showed comparable results to SVM and RF in model validation and consequently to the literature as cited above. However, when applying it to the same wild individuals the SVM and RF failed on, it classified a very different set of behaviours (Table 3). If we had only tested the two established approaches, we would have concluded that the transfer of a behaviour classification model trained on captive foxes to wild individuals is not possible. In contrast, the ANN with a moving window shows promising results that hopefully prompt further investigation into its potential use for wildlife ecology. The approach brings two additional advantages, besides the increased sample size: First, the likely better handling of recordings with mixed behaviours: By reducing the number of measurements per burst, the proportion of a potential second behaviour in the same burst is reduced. In some cases, this reduction may be enough to calculate similar summary statistics to a burst with only one of the behaviours. Second, the introduction of an ensemble learning effect: In case of a specific behaviour being performed in an unusual way or a burst containing more than one behaviour, it will be harder to infer the correct behaviour. As the moving window creates 32 subsamples of the original burst, using a majority vote for the resulting 32 assigned behaviours can reduce the uncertainty of the assignments. This framework could be a useful tool for future studies in wildlife research, especially for the study of species that are rarely kept in zoos or the study of behaviours that are hard to observe even in captivity.

The best size for the moving window was determined based on the maximal performance and slope of the GAM fit in the simulation plot (Fig 3). Our aim was to find a window size with a high mean performance that is small enough for generating sufficient data. Unfortunately, reducing the window size was found to negatively impact model performance [15]. Performance seemed to be at its maximum at window size 79. Larger windows would result in fewer subsets at worse performance, while performance also decreased for window sizes smaller than 79. Considering the slope of the GAM fit, the performance changed only marginally at window sizes 78 and 80 compared to 79. Therefore, we expect the model to perform similarly well at these window sizes. We suggest considering the slope because this approach may not always show a clear maximum like in our case. In cases of multiple maxima or plateau formations, the slope will help to inform on the smallest window size with the best performance.

A problem that may occur with the moving window approach is incorrect classification through overfitting [41]. By creating several similar subsets of the same burst, the ANN could build a model that fits the training data too well, i.e. even slight differences between training data and new data of the same behaviour class would result in the classification of different behaviours, with an overfitted model. Variation within the behaviour classes could also cause incorrect classifications if a single behaviour is realised outside the normal variation. There is no clear method to distinguish between these two causes of incorrect classifications. However, in the following we discuss the credibility of the assigned wild fox behaviour and argue that the moving window approach does not introduce overfitting.

Output credibility

The classification results of the classic methods appear to be obviously incorrect. At first glance, the output of the ANN appears more plausible than the output of RF and SVM, on account of all six behaviours getting classified. Still, the actual accuracy cannot be determined as wild individuals could not be observed. Since this may be true for most tagged wildlife, we provide four strategies to indirectly assess the credibility of the ANN output.

When looking at the time-dependent composition of behaviours, they appear quite consistent over individuals and time. Generally, some variation between individuals is apparent and some behaviour events seem to be misclassified. Individual differences in moving behaviour, for example, may result in the assignment of either walking or trotting when the algorithm is not accurate enough. However, an overall pattern is evident for most of the foxes and the temporal distribution appears plausible: The ANN output suggests that the foxes predominantly rest during the day and are active at night as well as during twilight (Fig 4, S1–S8 Figs) which corresponds well with described nocturnal-crepuscular activity patterns of red foxes (e.g. [42, 43]).

There also seem to be seasonal changes in these patterns, with fewer resting events during dark hours in the summer months. Although there are only two complete year-round datasets available, this pattern appears reasonable as the nights in summertime at this longitude are much shorter than during winter months (in Berlin, the daily dark period ranges from 7 to 17 hours during the course of a year). Thus, foxes should use the full night-time spectrum in summer for their activities, while during winter the higher availability of potential activity time allows nocturnal resting events. This behavioural plasticity in activity has also been shown by Ricci et al. [44] who found that foxes are active in different zones and hours of the day, according to the season.

Like trotting and walking, feeding is mostly classified at night-time. Feeding events occur in no clustered manner. The mixture of movement and feeding events reflects the feeding ecology of foxes which do not feed on large prey. Foxes mostly hunt for small prey like mice and voles and often rather scavenge than hunt, especially in urbanized areas [45, 46].

While the behaviour composition is very similar across all individuals we cannot rule out the possibility that the wild foxes performed a behaviour that is very similar to another behaviour class that we did not observe in the captive foxes. Consequently this behaviour class would have been misclassified as one of the behaviour classes that we did observe in the captive foxes. Due to the nature of our study we do not have the possibility to test whether that was the case or not because we do not have access to the actual behaviour of the wild foxes. In any case, this behaviour class or classes would have to be behaviour that are universal in wild foxes but are not or very rarely performed by captive foxes. Obtaining observation and acceleration data of such behaviour for the training of any machine learning algorithm would be quite challenging. Generally, similar results do necessarily indicate correct predictions, but dissimilarities between individuals could hint to poor model performance. Anyhow, the credibility of the output has to be addressed by using different approaches. Therefore we also used GPS data to relate the occurrence of GPS-clusters as well as GPS-based speed values to the assigned behaviour classes.

In particular, we focused on resting and movement behaviour (trotting and walking), with an obvious connection to be expected. Resting as a stationary behaviour should get classified predominantly at locations where GPS points are clustered (Fig 5). We found that 38% to 74% of resting bursts were located within such a cluster. The remaining bursts may reflect cases when the individual had just temporarily stopped moving. Standing still or sitting briefly during an active phase would also be classified as resting but may not be associated with a GPS-cluster.

Trotting as a locomotive behaviour was expected to show low association with GPS-clusters and was only classified at a cluster for 2% to 9% of all bursts (Fig 5, S8–S14 Figs). As foxes move away from or to a resting site, it is reasonable for some trotting to be classified within GPS clusters. The analysis did not target feeding, caching or grooming, as those behaviours can be performed in a clustered or non-clustered manner. The analysis of behaviour assignments in relation to speed shows a reverse picture: Behaviours that show a weak association with GPS clusters show a higher speed and vice versa (S8–S14 Figs). However, we could use only 5% to 15% of all data for the speed analysis (S6 Table). The method described here is hence more applicable when the recording of location and acceleration data is better synchronised.

Finally, we analysed the ODBA, an indicator of body movement [18] that was shown to correspond well with the activity level of specific behaviours [15]. When we compared the temporal distribution of ODBA values to that of the classified trotting and resting events, we saw an association of high ODBA values and trotting behaviour and low ODBA values and resting behaviour, respectively. Again, the nocturnal-crepuscular activity pattern was visible (Fig 6, S15–S21 Figs).

While the above mentioned examples appear conceivable, the interpretation of some behaviours may be puzzling, and their biological credibility is difficult to gauge. For instance, we could not identify any pattern for caching behaviour, and grooming seems to be generally over-classified. Its complexity and the resulting variability in the training data set may increase misclassification of unknown behaviours, especially when considering that six behaviours do not represent the full variety of behaviour that this mobile carnivore displays in the wild. The latter is clearly more significant for fast-moving animals like the highly agile red fox that displays a variety of complex movement patterns. Generating as precise outputs as they, for example, have been shown for grazing animals with their limited body flexibility and behavioural repertoire (see [28, 47]), therefore remains a challenge. In another study on captive red foxes Painter et al. [48] could classify three exhibited behaviours with an accuracy of 95.7% when training on one individual, and predicted the behaviour of a second individual at an accuracy of 66.7%, suggesting that the classifier can extract behaviours across multiple foxes.

In the present study, a broader training dataset of more captive individuals could possibly improve the output of the ANN for the wild foxes and permit more precise recognition of specific behaviours. However, our results suggest that the behaviour inferred by the ANN corresponds well with the actual behaviour of the logged foxes. Despite some unsolved issues, the ANN thus seems to be a promising approach to infer wildlife behaviour, even in cases where methods suggested by existing literature fail.

Conclusion

We here compare the relative predictive power of different machine learning approaches in inferring wildlife behaviour and we could show that good results for the validation of the models will not necessarily lead to good results when these models are applied in the field. We provide a framework to use acceleration data and an Artificial Neural Network to infer the behaviour of wild foxes, using a training data set obtained from captive individuals. We also present four strategies to address the plausibility of such behaviour classification output when no direct validation is possible. Although not all validation strategies may be applicable for every species, this framework should not be restricted to the studied species. The successful application of the ANN for behavioural classification on field data offers exciting potential to study the behaviour of animals in the wild without direct observation.

Supporting information

S1 Fig. Time-dependent composition of behaviours of fox “Gisel”. Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from September 2016 until March 2017. Plots for September and March are only based on 2 and 7 days, respectively, and cannot be interpreted. Because of a logger failure the February plot is only based on 12 days and should also not be considered. Gisel shows more walking and feeding than most other individuals.
(TIF)

S2 Fig. Time-dependent composition of behaviours of fox “Hazel”. Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from October 2016 until February 2017. Hazel shows more feeding than most other individuals.
(TIF)

S3 Fig. Time-dependent composition of behaviours of fox “Ida”. Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from November 2016 until April 2017. Ida shows almost no walking.
(TIF)

S4 Fig. Time-dependent composition of behaviours of fox “Jack”. Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from January 2017 until December 2017. The plot for January is only based on 7 days and should not be interpreted. Jack shows much more grooming than all other individuals, as well

as much less resting.
(TIF)

S5 Fig. Time-dependent composition of behaviours of fox "Kyna". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from February 2017 until November 2017. The logger failed between the 15th of June and the 26 of July. The June plot looks jagged because of the resulting lack of data. The July plot cannot be interpreted because it only relies on 6 days. Generally, less trotting is predicted for Kyna compared to the other foxes.
(TIF)

S6 Fig. Time-dependent composition of behaviours of fox "Nikita". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from September 2017 until January 2018. The September and January plots cannot be interpreted because both are based on 5 days only. For this fox much more walking than trotting is predicted. In addition, much more feeding is predicted than for most other individuals.
(TIF)

S7 Fig. Time-dependent composition of behaviours of fox "Porthos". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from October 2017 until June 2018. The logger failed for 15 days in March and for 8 days in April. The March plot therefore shows only half as much data as the other plots and looks more jagged. The October plot is only based on 6 days and can thus not be interpreted properly.
(TIF)

S8 Fig. Behaviour assignments of "Gisel" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (57%) and trotting the lowest (2%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, $W = 727995$, $p < 0.001$).
(TIF)

S9 Fig. Behaviour assignments of "Hazel" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (38%) and trotting the lowest (9%). Resting is predicted much less in clusters than in most other study foxes. (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, $W = 489223$, $p < 0.001$).
(TIF)

S10 Fig. Behaviour assignments of "Ida" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (46%) and trotting the lowest (9%). Resting is predicted much less in clusters than in most other study foxes. (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, $W = 803283$, $p < 0.001$).
(TIF)

S11 Fig. Behaviour assignments of "Jack" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (74%) and trotting the lowest (6%). Resting is

predicted much less in clusters than in most other study foxes. (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 295149, $p < 0.001$).
(TIF)

S12 Fig. Behaviour assignments of "Kyna" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (57%) and trotting the lowest (3%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 428274, $p < 0.001$).
(TIF)

S13 Fig. Behaviour assignments of "Nikita" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (69%) and trotting the lowest (8%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 327626, $p < 0.001$).
(TIF)

S14 Fig. Behaviour assignments of "Porthos" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (68%) and trotting the lowest (8%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 4454986, $p < 0.001$).
(TIF)

S15 Fig. Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Gisel". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime. Discontinuities in the red line are caused by missing data due to the logger not recording data at the time.
(TIF)

S16 Fig. Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Hazel". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.
(TIF)

S17 Fig. Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Ida". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours.

Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.
(TIF)

S18 Fig. Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Jack". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.
(TIF)

S19 Fig. Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Kyna". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime. Discontinuities in the red line are caused by missing data due to the logger not recording data at the time.
(TIF)

S20 Fig. Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Nikita". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.
(TIF)

S21 Fig. Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Porthos". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime. Discontinuities in the red line are caused by missing data due to the logger not recording data at that time.
(TIF)

S1 Table. Ethogram for the captive foxes—Modified from [16]. Behavioural observations were restricted to the listed categories. Each burst could only have one behaviour assigned. Observations not matching any description as well as observations of more than one behaviour per burst were excluded from the analysis.
(DOCX)

S2 Table. List of all predictors and how they were calculated. References refer to when the respective predictor was first introduced in the context of behaviour prediction. All predictors except the Fast Fourier Transformation can be considered summary statistics because they result in a single number. We add the complete Fast Fourier Spectrum as predictors. The total amount of predictors from the Fast Fourier Spectrum is therefore dependant on burst length.
(DOCX)

S3 Table. Confusion matrix for the support vector machine (SVM) validation. Columns show expected behaviours known from observation, rows show behaviours assigned by the SVM. Values on the diagonal (bold) represent behaviours assigned correctly. All values off the diagonal are incorrect assignments that show which behaviours were confused with each other (for example 16 events of feeding were incorrectly classified as grooming).
(DOCX)

S4 Table. Confusion matrix for the random forest (RF) validation. Columns show expected behaviours known from observation, rows show behaviours assigned by the RF. Values on the diagonal (bold) represent behaviours assigned correctly. All values off the diagonal are incorrect assignments that show which behaviours were confused with each other (e.g. 17 events of feeding were incorrectly classified as grooming).
(DOCX)

S5 Table. Confusion matrix for the artificial neural network (ANN) validation. Columns show expected behaviours known from observation, rows show behaviours assigned by the ANN. Values on the diagonal (bold) represent behaviours assigned correctly. All values off the diagonal are incorrect assignments that show which behaviours were confused with each other (for example 15 events of resting were incorrectly predicted as feeding).
(DOCX)

S6 Table. Proportion of data used for the speed analysis. Most of the times GPS and acceleration data were not recorded simultaneously. For the speed analysis we considered only acceleration data that was recorded within 10 seconds of a GPS recording.
(DOCX)

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Supporting Information for: Machine learning goes wild: Using data from captive individuals to infer wildlife behaviour.

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Table of content

Tables

Table S1: Ethogram for the captive foxes - modified from (Giese, 2016). Behavioural observations were restricted to the listed categories. Each burst could only have one behaviour assigned. Observations not matching any description as well as observations of more than one behaviour per burst were excluded from the analysis.

Behaviour	**Description**
feeding	licking food items while standing on all four legs with the head held down; chewing on food items while sitting on the hind legs with head held down or on level with shoulders; chewing on food items while standing on all four legs with the head held down or head on level with shoulders
grooming	licking the body while lying on the stomach with head held up; licking the body while sitting with the head held down; scratching body parts with one hind leg while lying on the stomach with the head held up; scratching body parts while sitting with the head held up or on level with shoulders; nibbling body parts while lying on the stomach with head held up; nibbling body parts while sitting on hind legs with head held up, on level with shoulders or held down; nibbling body parts while standing on all four legs with the head held up or down
resting	lying on the stomach with the head resting on the ground; lying on the stomach with the head held up, motionless or slightly moving from side to side; sitting on hind legs with the head held up motionless or slightly moving from side to side; standing on all four legs with the head held up motionless or slightly moving from side to side
caching	digging into the ground using one or both forelegs while standing on all four legs with the head held down covering food items with soil and occasionally pressing it down using the snout while standing on all four legs with the head held down
trotting	moderately fast locomotion with all four feet losing contact with the ground for short moments with the head held up or on level with shoulders
walking	slow locomotion with at least three feet touching the ground at any given moment with the head on level with shoulders or held down and moving from side to side

Table S2: List of all predictors and how they were calculated. References refer to when the respective predictor was first introduced in the context of behaviour prediction. All predictors except the Fast Fourier Transformation can be considered summary statistics because they result in a single number. We add the complete Fast Fourier Spectrum as predictors. The total amount of predictors from the Fast Fourier Spectrum is therefore dependant on burst length.

Predictor	**Calculation**	**Reference**
Mean	mean of the x axis x mean of the y axis y mean of the z axis z	
Standard deviation (sd)	sd of the x axis sd of the y axis sd of the z axis	
Inverse Coefficient of Variation (ICV)	xsdofthexaxis ysdoftheyaxis zsdofthezaxis	
Variation	1n−1sd^{2}ofthexaxis 1n−1sd^{2}oftheyaxis 1n−1sd^{2}ofthezaxis	
Skewness	skewness() function in R, type = 3 of the x axis skewness() function in R, type = 3 of the y axis skewness() function in R, type = 3 of the z axis	(Meyer et al., 2017)
Kurtosis	kurtosis() function in R, type = 3 of the x axis kurtosis() function in R, type = 3 of the y axis kurtosis() function in R, type = 3 of the z axis	(Meyer et al., 2017)
q	x2+y2+z2	(Nathan et al., 2012)
Pitch	arctanyx2+z2180	(Collins et al., 2015)
Roll	arctanxy2+z2180	(Collins et al., 2015)
Overall Body acceleration (ODBA)	1nxn−x+1nyn−y+1nzn−z	(Wilson et al., 2006)
Fast Fourier Transformation (FFT)	fft() function in R of the x axis considering only the real numbers fft() function in R of the y axis considering only the real numbers fft() function in R of the z axis considering only the real numbers	(R Core Team, 2018)

Table S3: Confusion matrix for the support vector machine (SVM) validation. Columns show expected behaviours known from observation, rows show behaviours assigned by the SVM. Values on the diagonal (bold) represent behaviours assigned correctly. All values off the diagonal are incorrect assignments that show which behaviours were confused with each other (for example 16 events of feeding were incorrectly classified as grooming).

	Expected					
Assigned	**feeding**	**grooming**	**resting**	**caching**	**trotting**	**walking**
feeding	**47**	30	0	3	0	1
grooming	1	**114**	48	0	0	0
resting	1	7	**565**	1	0	1
caching	27	41	6	**22**	0	10
trotting	32	136	9	32	**54**	24
walking	2	14	6	1	0	**13**

Table S4: Confusion matrix for the random forest (RF) validation. Columns show expected behaviours known from observation, rows show behaviours assigned by the RF. Values on the diagonal (bold) represent behaviours assigned correctly. All values off the diagonal are incorrect assignments that show which behaviours were confused with each other (e.g. 17 events of feeding were incorrectly classified as grooming).

	Expected					
Predicted	**feeding**	**grooming**	**resting**	**caching**	**trotting**	**walking**
feeding	**77**	10	1	13	0	7
grooming	20	**319**	47	2	2	9
resting	0	5	**584**	1	0	1
caching	12	6	1	**40**	0	9
trotting	1	0	0	2	**52**	2
walking	0	2	1	1	0	**21**

Table S5: Confusion matrix for the Artificial Neural network (ANN) validation. Columns show expected behaviours known from observation, rows show behaviours assigned by the ANN. Values on the diagonal (bold) represent behaviours assigned correctly. All values off the diagonal are incorrect assignments that show which behaviours were confused with each other (for example 15 events of resting were incorrectly predicted as feeding).

	Expected					
Predicted	**feeding**	**grooming**	**resting**	**caching**	**trotting**	**walking**
feeding	**2934**	252	19	199	0	86
grooming	198	**9643**	237	78	0	34
resting	15	282	**19548**	1	0	54
caching	103	62	32	**1287**	47	22
trotting	0	47	1	33	**1656**	88
walking	84	228	36	123	8	**1157**

Table S6: Proportion of data used for the speed analysis. Most of the times GPS and acceleration data were not recorded simultaneously. For the speed analysis we considered only acceleration data that was recorded within 10 seconds of a GPS recording.

Individual	**Proportion of data used**
Porthos	0.15
Kyna	0.08
Nikita	0.15
Gerlinde	0.05
Gisel	0.11
Hazel	0.10
Ida	0.11
Jack	0.09
Que	0.07

Figures

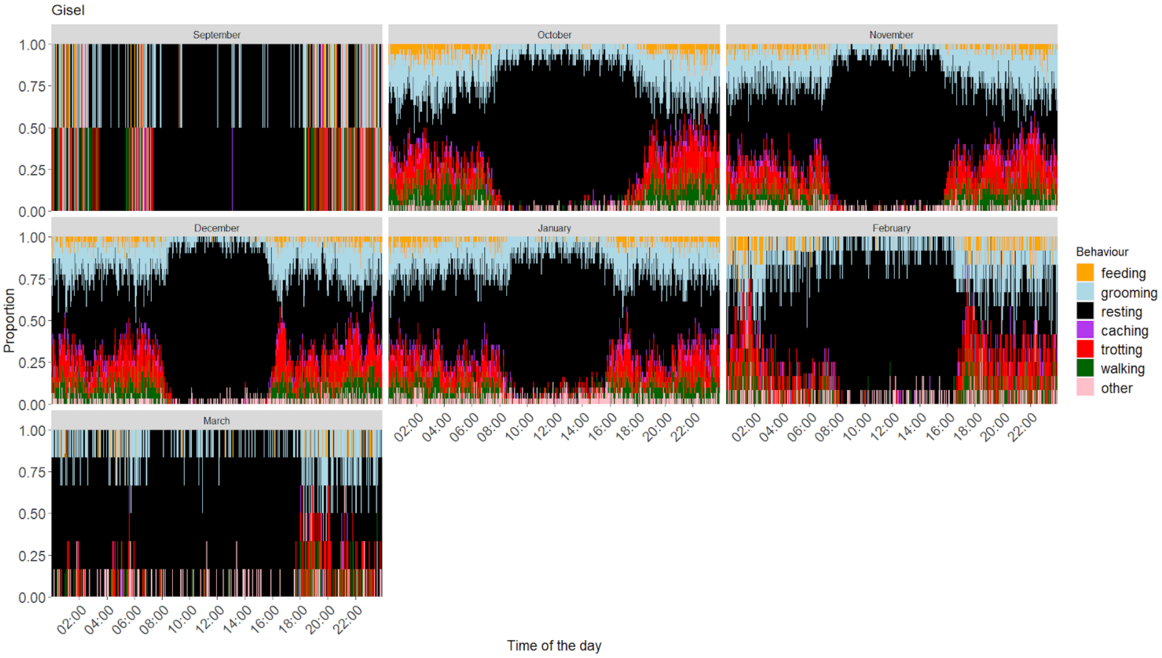

Figure S1: Time-dependent composition of behaviours of fox "Gisel". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from September 2016 until March 2017. Plots for September and March are only based on 2 and 7 days, respectively, and cannot be interpreted. Because of a logger failure the February plot is only based on 12 days and should also not be considered. Gisel shows more walking and feeding than most other individuals.

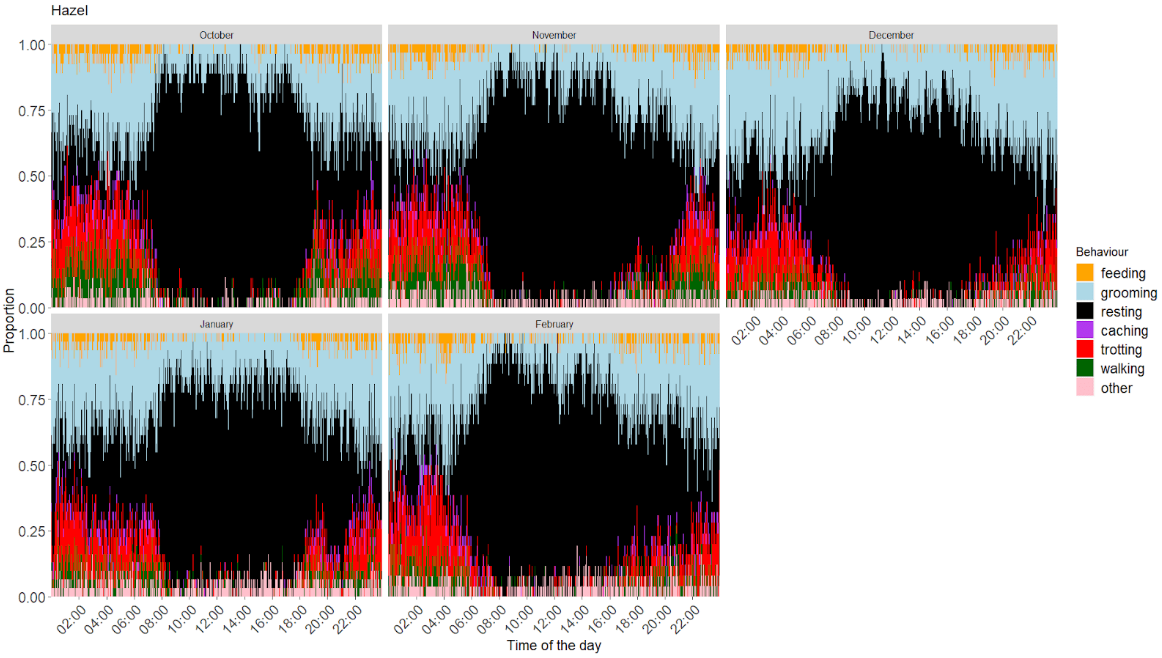

Figure S2: Time-dependent composition of behaviours of fox "Hazel". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from October 2016 until February 2017. Hazel shows more feeding than most other individuals.

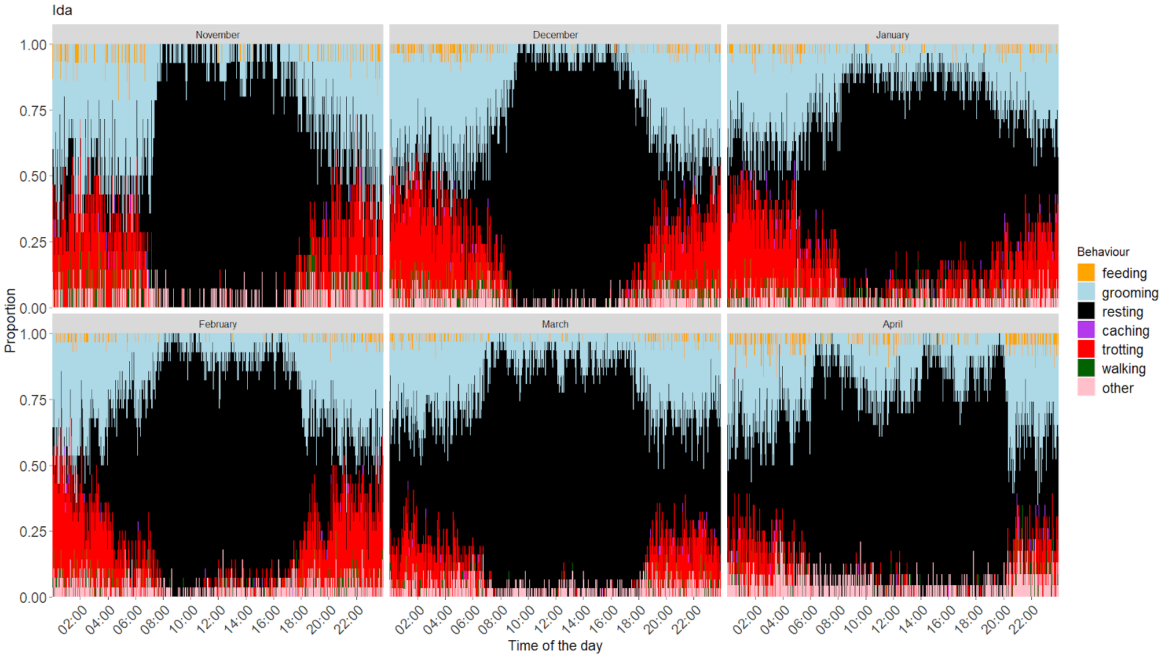

Figure S3: Time-dependent composition of behaviours of fox "Ida". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from November 2016 until April 2017. Ida shows almost no walking.

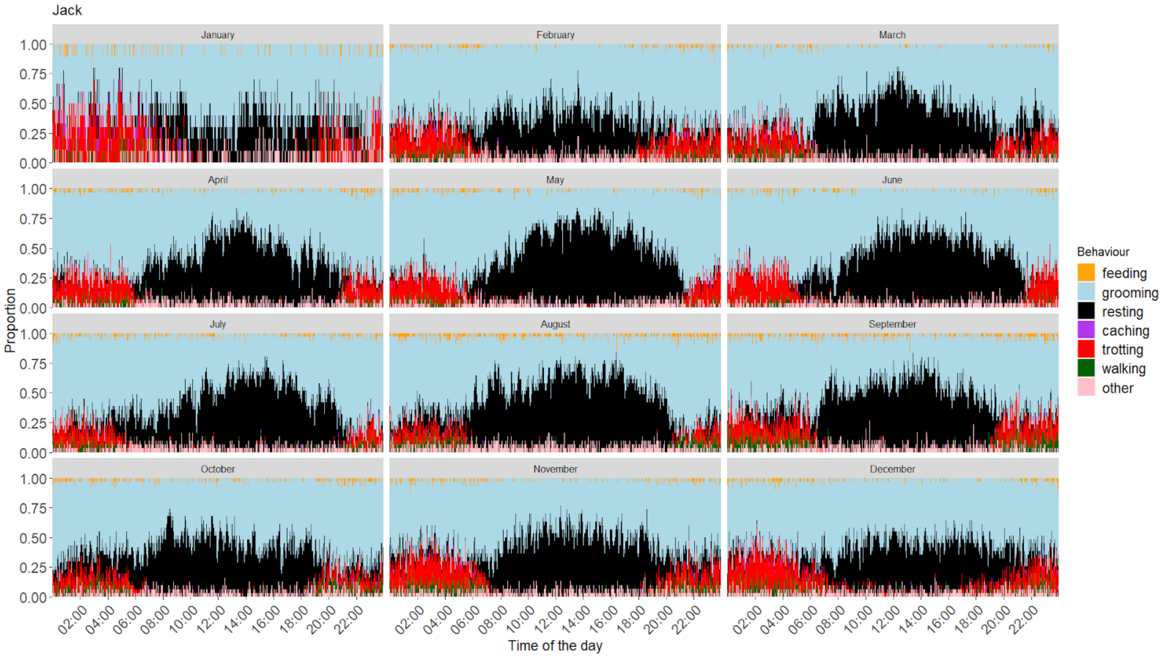

Figure S4: Time-dependent composition of behaviours of fox "Jack". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from January 2017 until December 2017. The plot for January is only based on 7 days and should not be interpreted. Jack shows much more grooming than all other individuals, as well as much less resting.

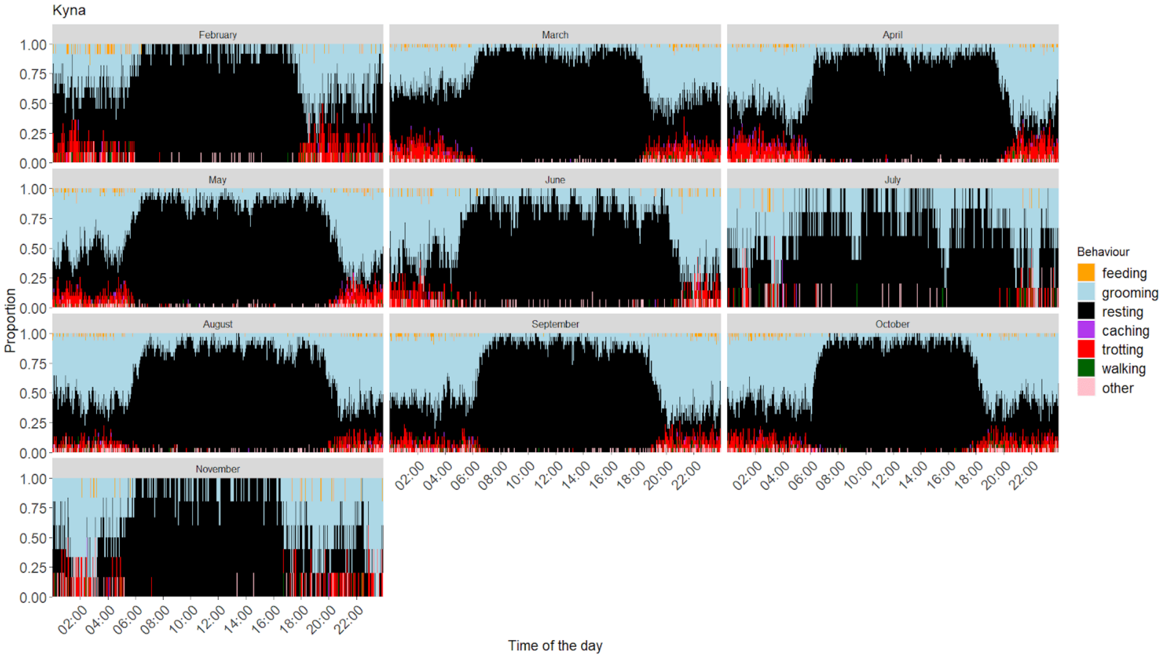

Figure S5: Time-dependent composition of behaviours of fox "Kyna". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from February 2017 until November 2017. The logger failed between the 15th of June and the 26 of July. The June plot looks jagged because of the resulting lack of data. The July plot cannot be interpreted because it only relies on 6 days. Generally, less trotting is predicted for Kyna compared to the other foxes.

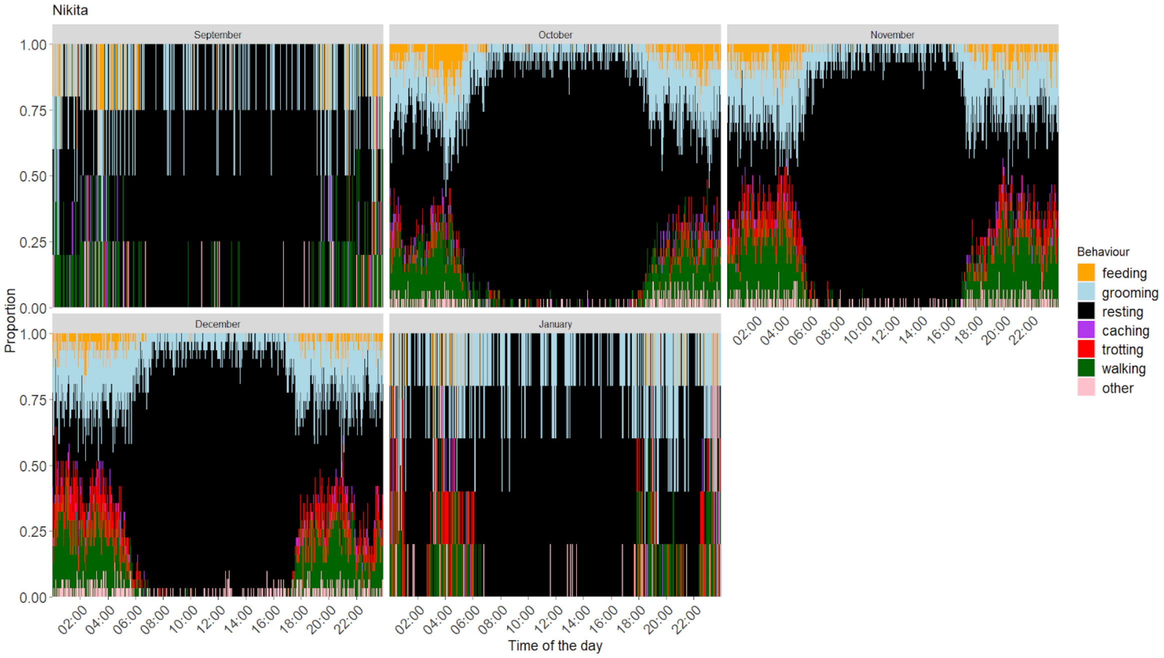

Figure S6: Time-dependent composition of behaviours of fox "Nikita". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from September 2017 until January 2018. The September and January plots cannot be interpreted because both are based on 5 days only. For this fox much more walking than trotting is predicted. In addition, much more feeding is predicted than for most other individuals.

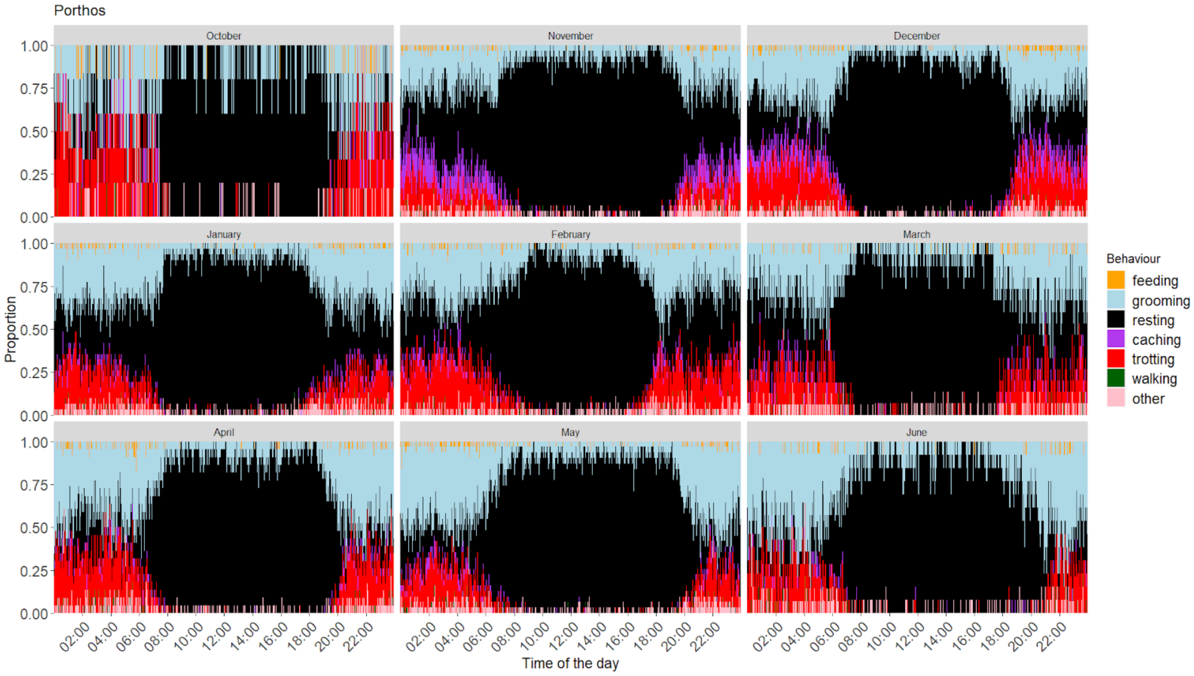

Figure S7: Time-dependent composition of behaviours of fox "Porthos". Stacked bars represent the proportion of each behaviour at a given time of day, in each month. The data shown here span from October 2017 until June 2018. The logger failed for 15 days in March and for 8 days in April. The March plot therefore shows only half as much data as the other plots and looks more jagged. The October plot is only based on 6 days and can thus not be interpreted properly.

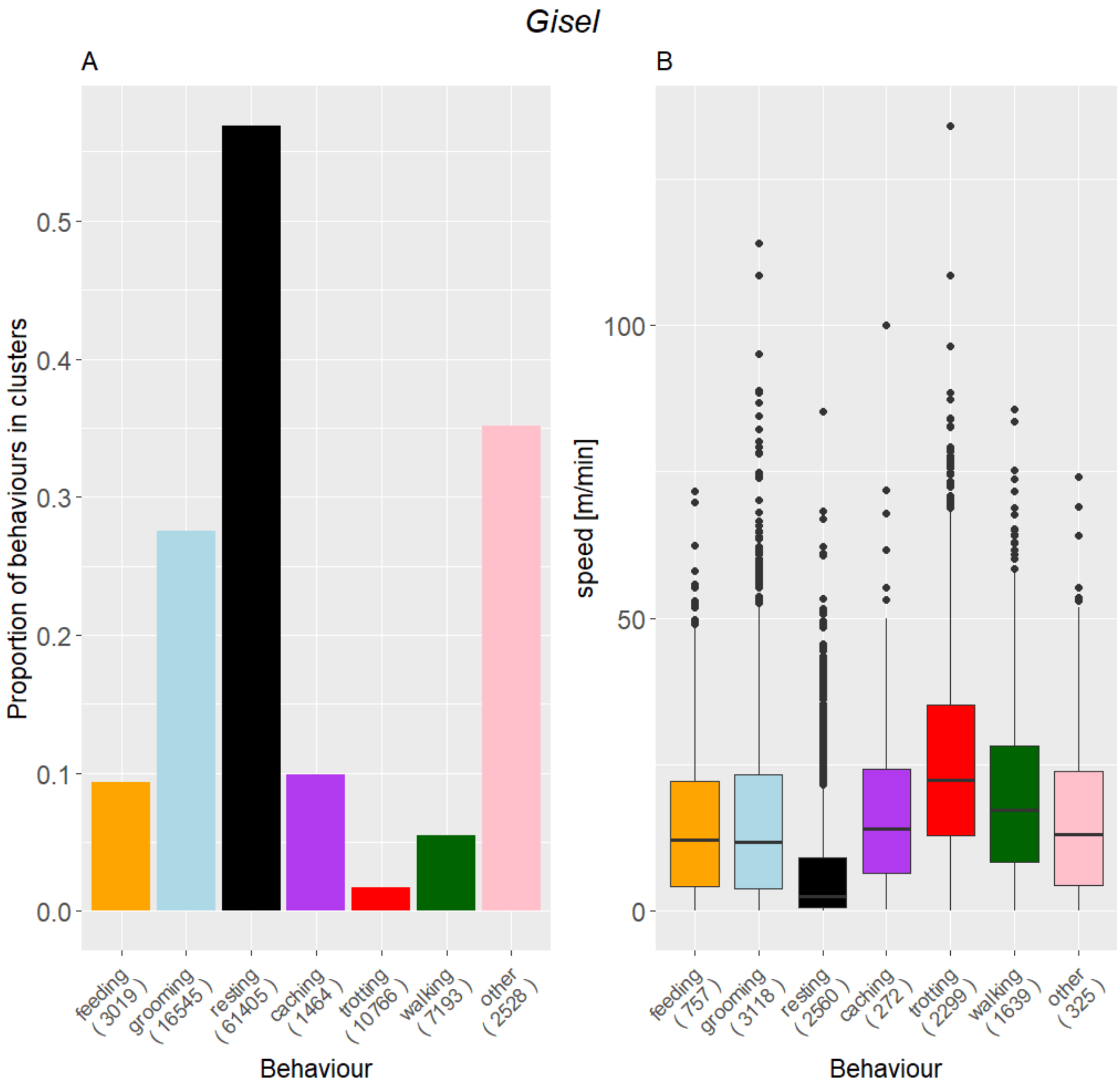

Figure S8: Behaviour assignments of "Gisel" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (57%) and trotting the lowest (2%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 727995, p < 0.001).

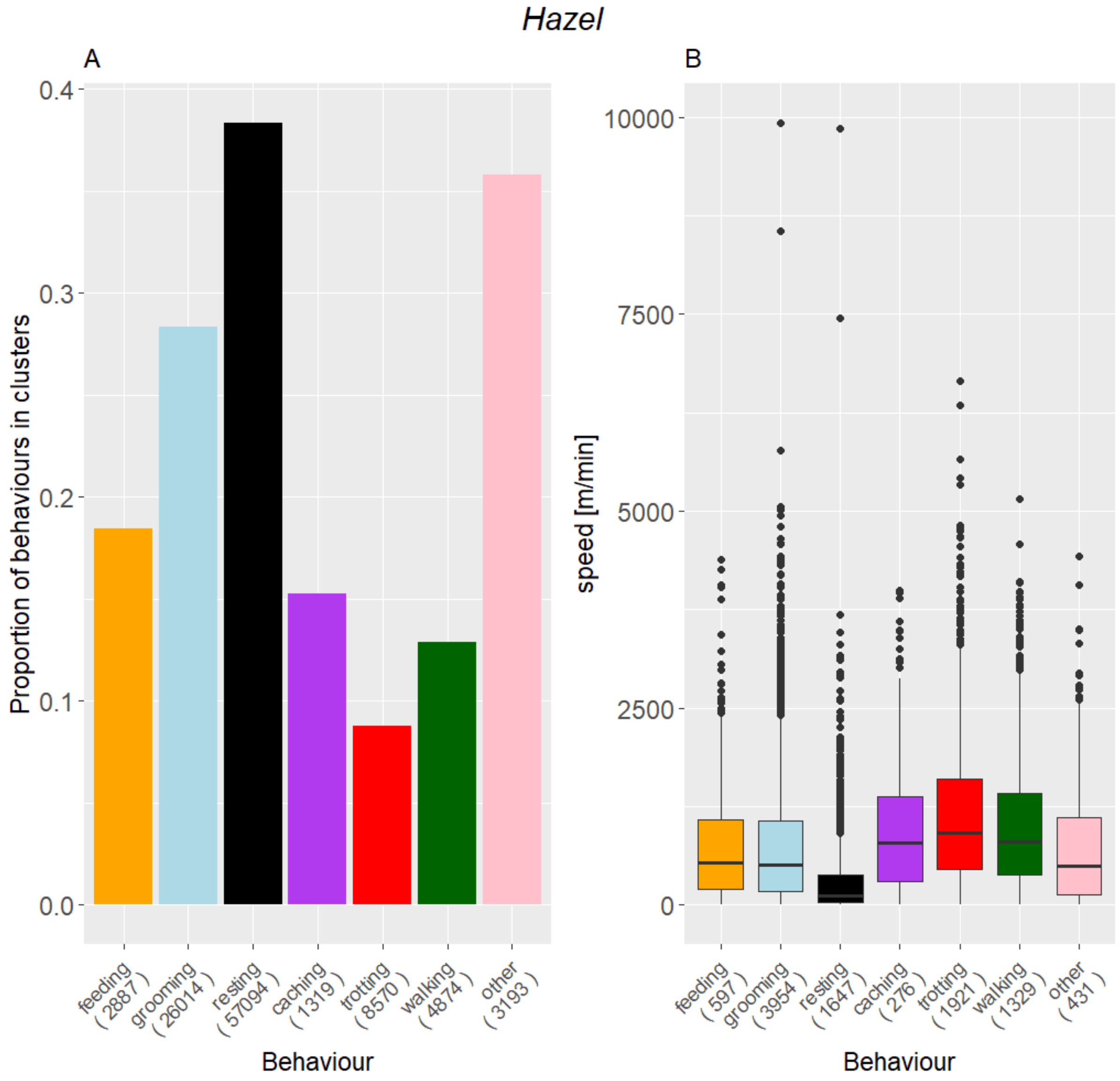

Figure S9: Behaviour assignments of "Hazel" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (38%) and trotting the lowest (9%). Resting is predicted much less in clusters than in most other study foxes. (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 489223, $p < 0.001$).

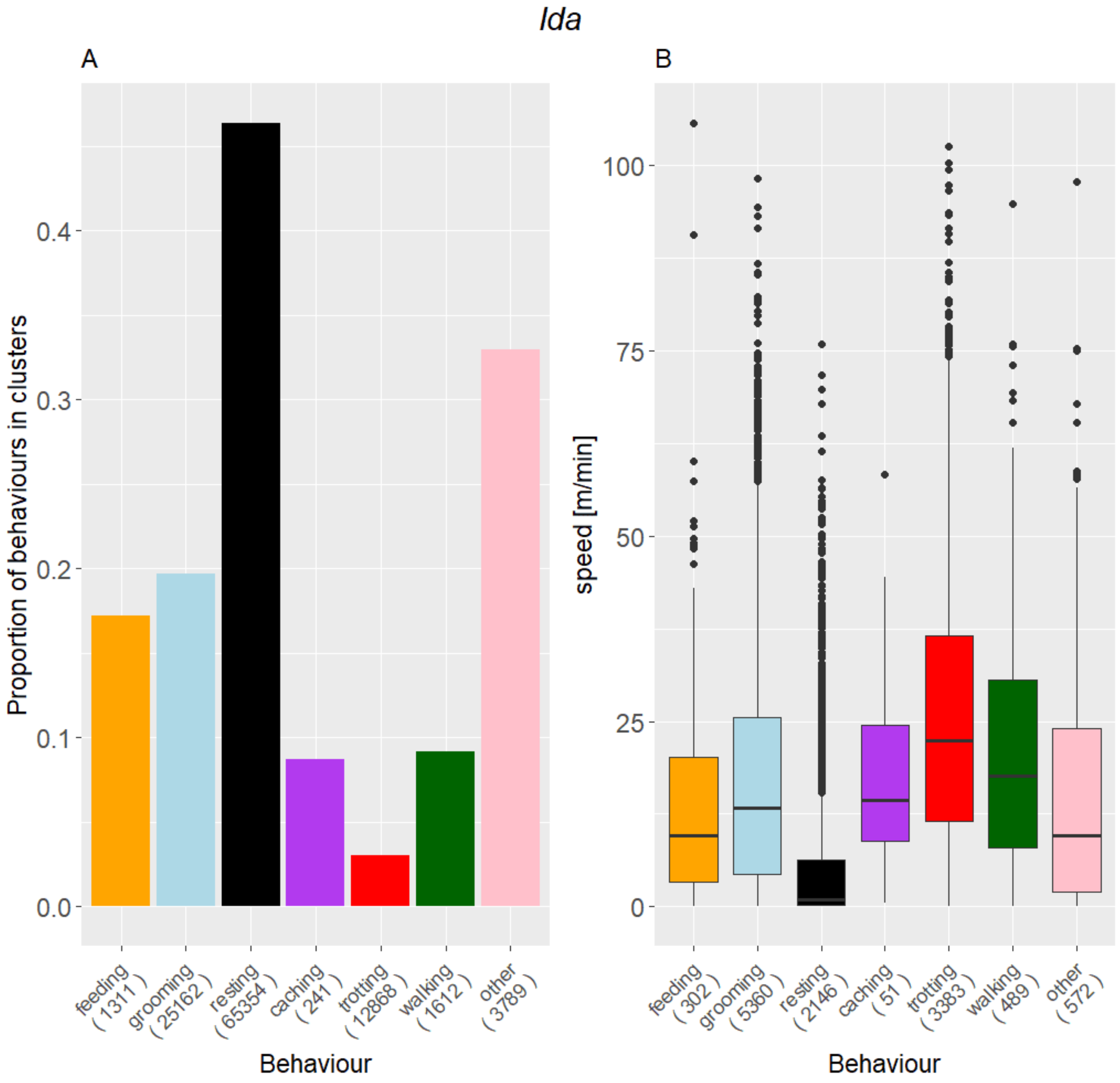

Figure S10: Behaviour assignments of "Ida" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (46%) and trotting the lowest (9%). Resting is predicted much less in clusters than in most other study foxes. (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 803283, $p < 0.001$).

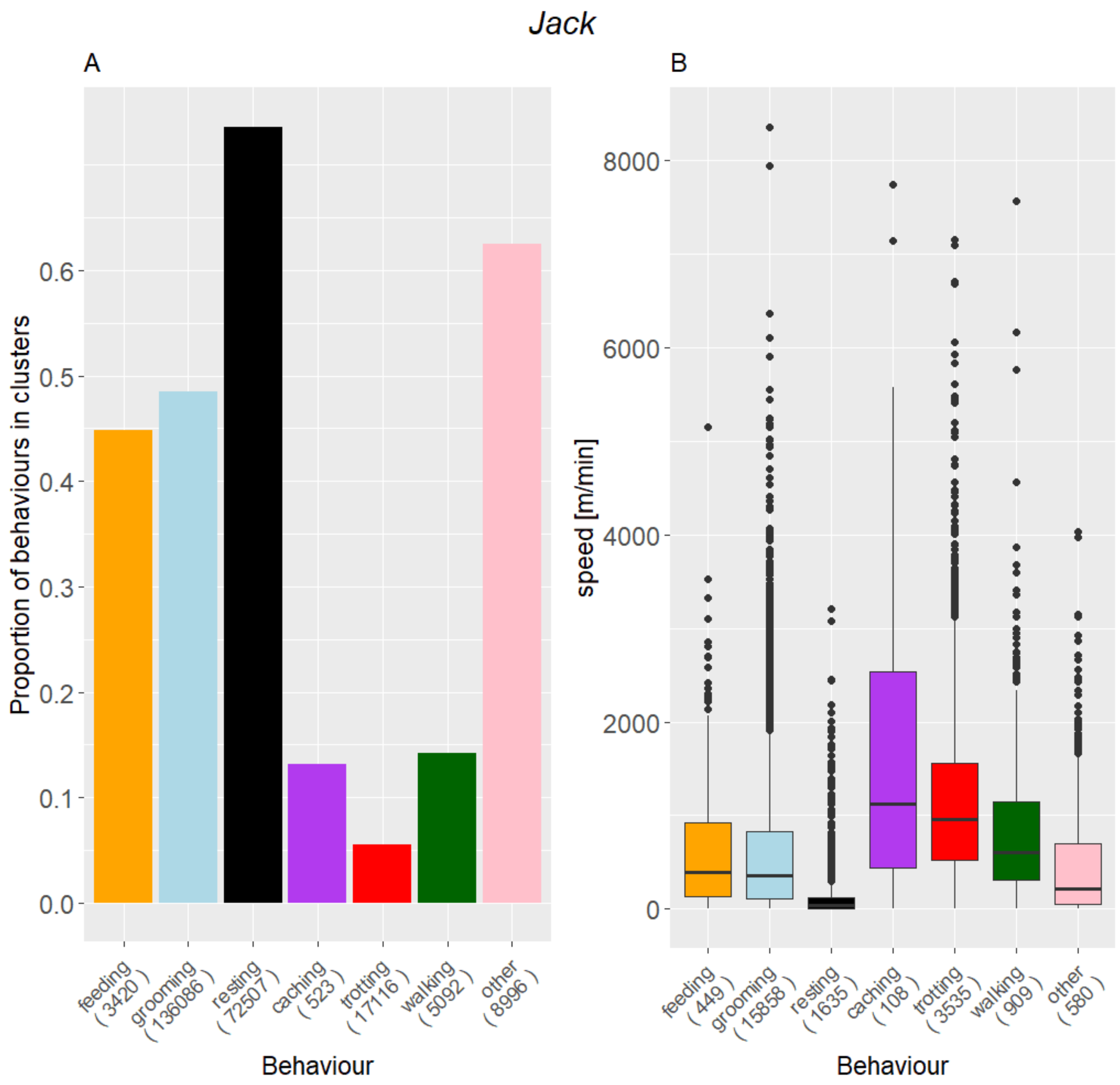

Figure S11: Behaviour assignments of "Jack" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (74%) and trotting the lowest (6%). Resting is predicted much less in clusters than in most other study foxes. (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 295149, p < 0.001).

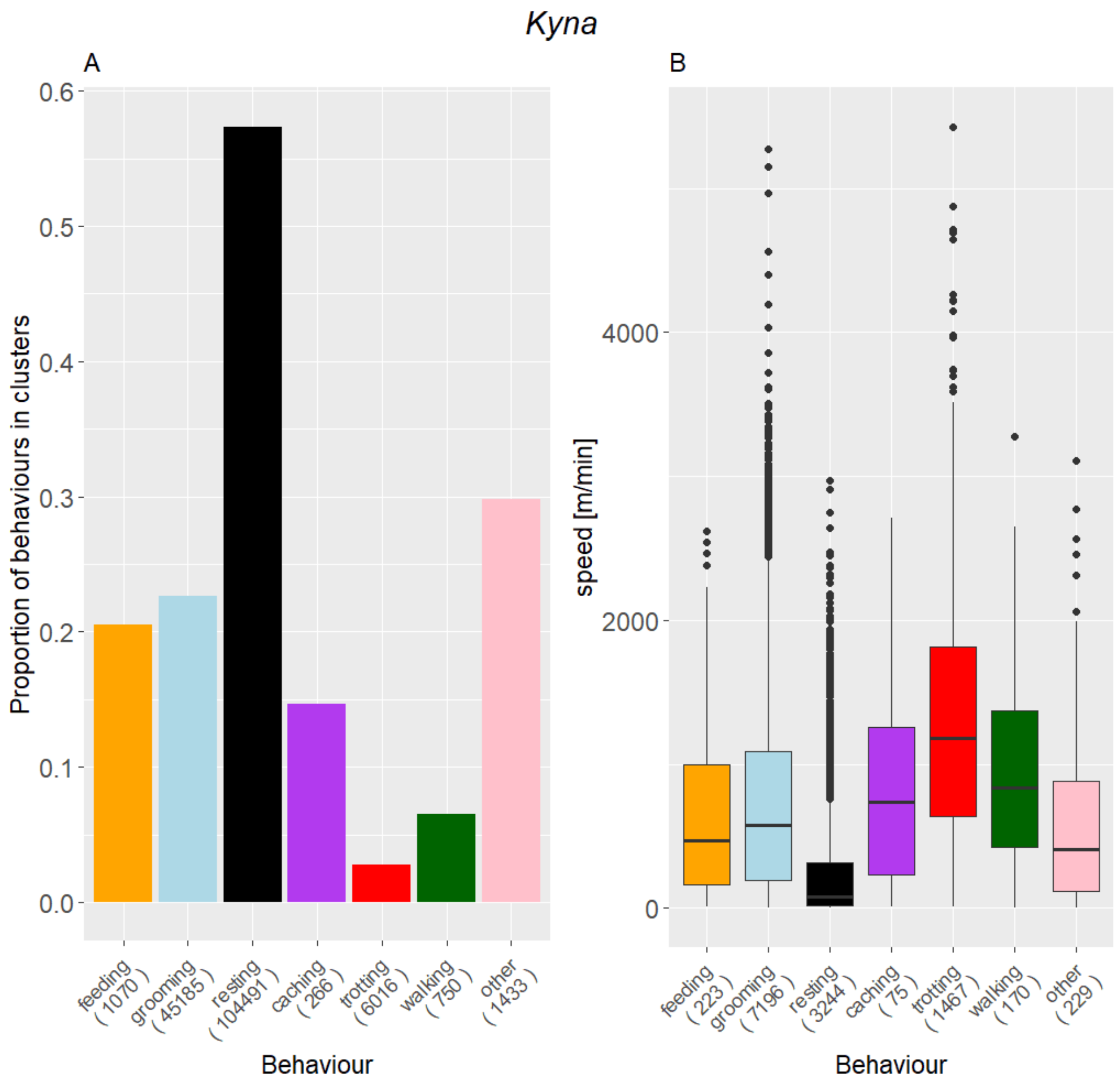

Figure S12: Behaviour assignments of "Kyna" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (57%) and trotting the lowest (3%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 428274, $p < 0.001$).

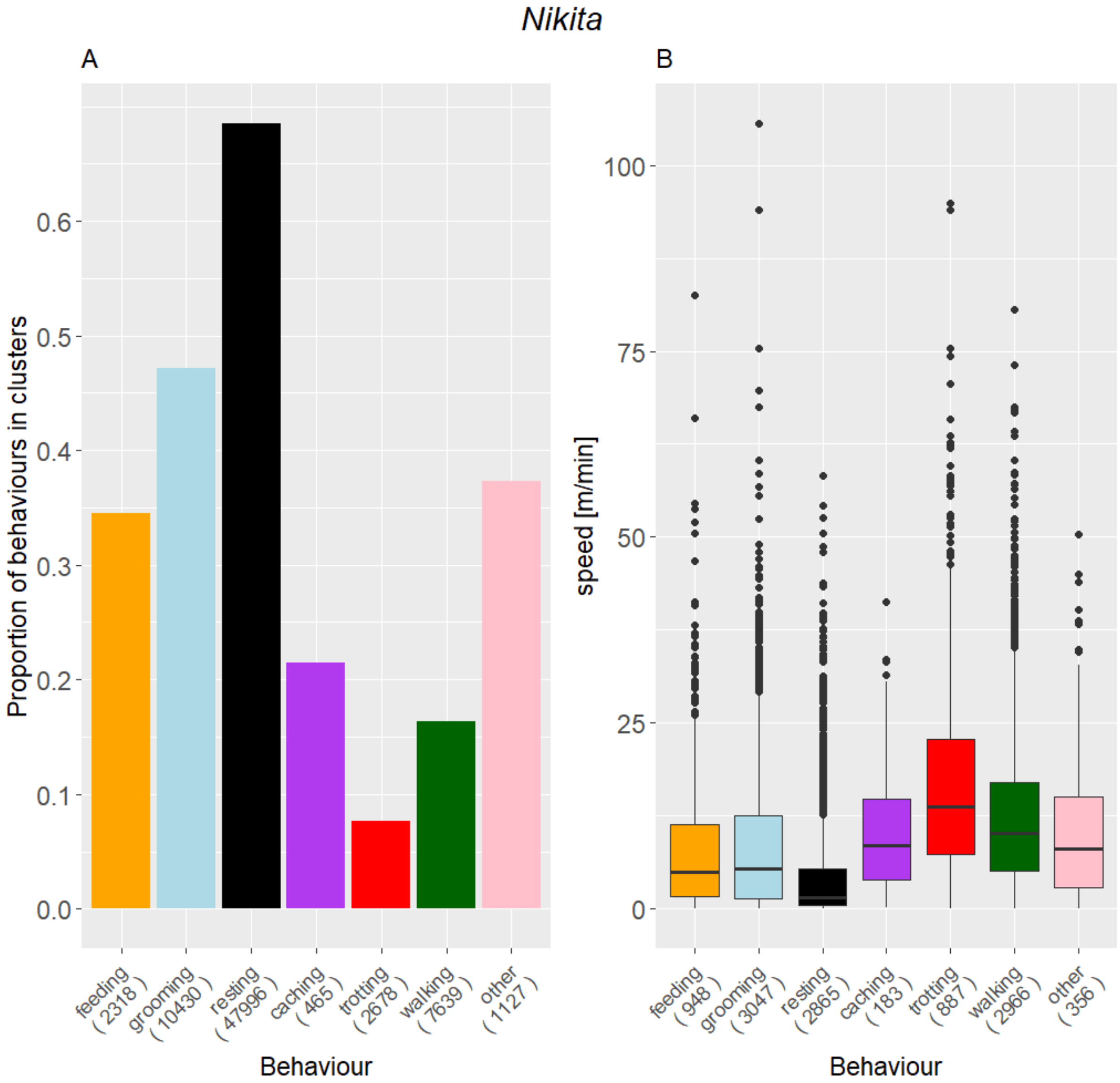

Figure S13: Behaviour assignments of "Nikita" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (69%) and trotting the lowest (8%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, W = 327626, p < 0.001).

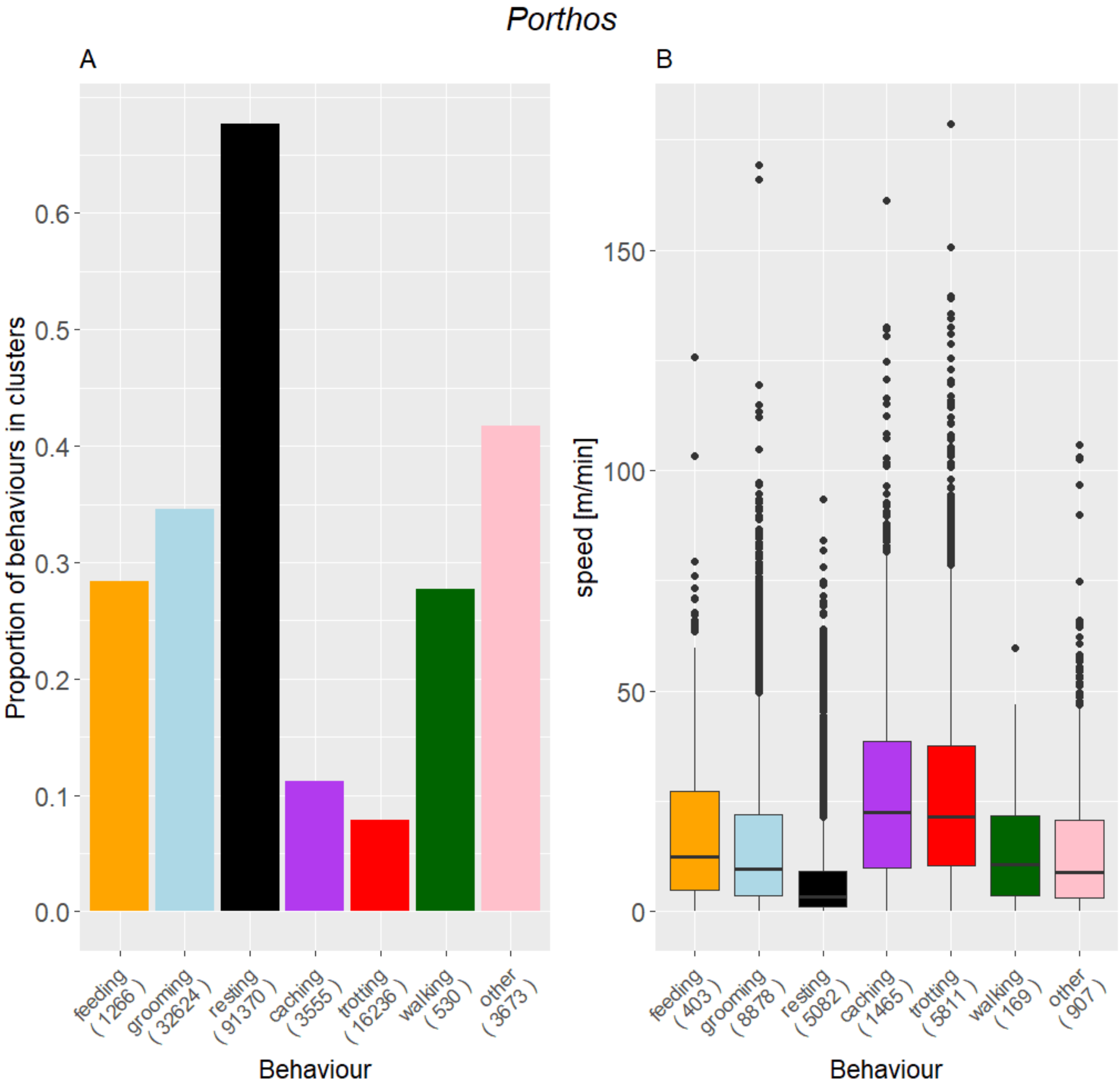

Figure S14: Behaviour assignments of "Porthos" in relation to GPS clusters (A) and speed (B). The numbers in the brackets indicate the sample size of each behaviour class. (A) Resting shows the highest association with GPS clusters (68%) and trotting the lowest (8%). (B) Behaviour prediction in relation to speed. Resting appears at significantly lower speeds than trotting (Wilcoxon rank sum test, $W = 4454986$, $p < 0.001$).

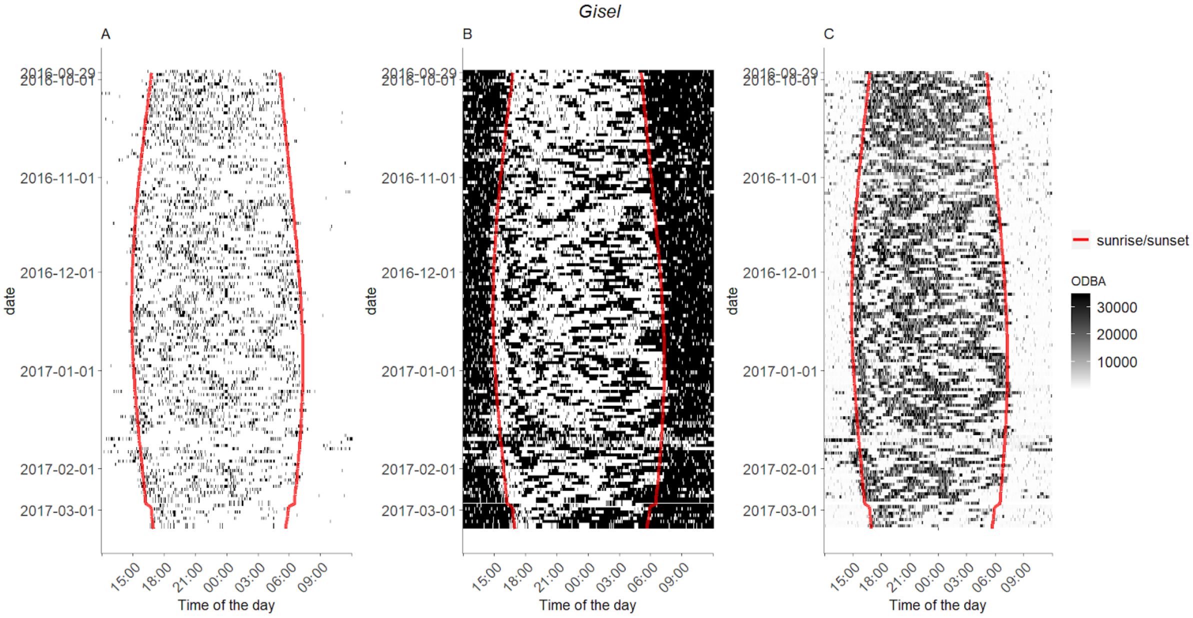

Figure S15: Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Gisel". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime. Discontinuities in the red line are caused by missing data due to the logger not recording data at the time.

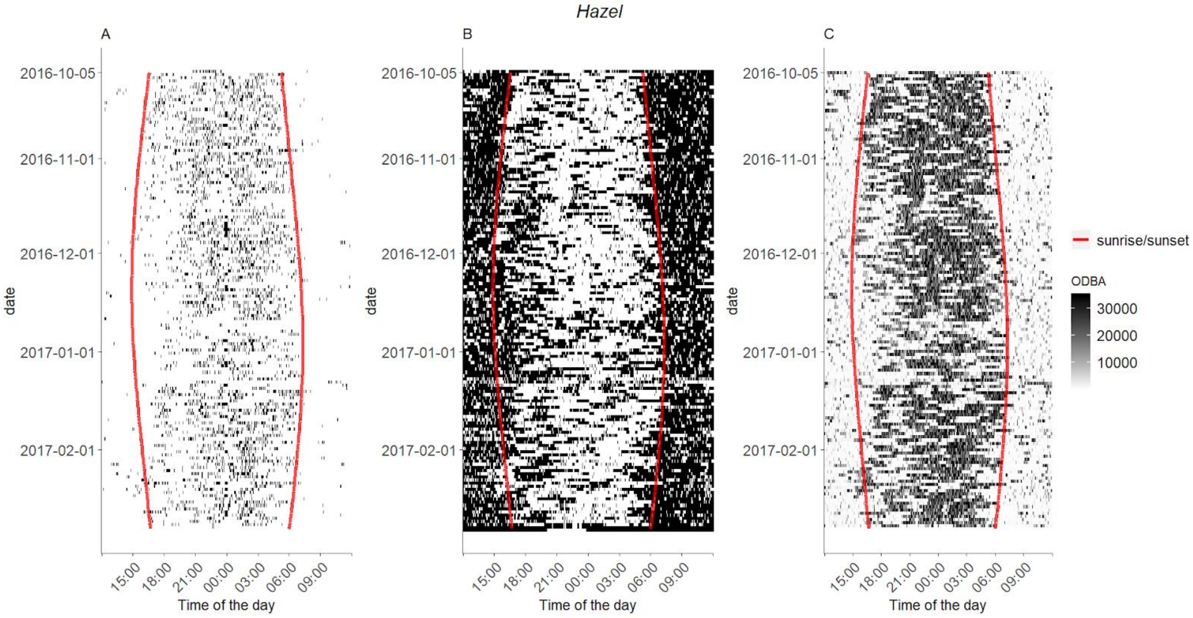

Figure S16: Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Hazel". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.

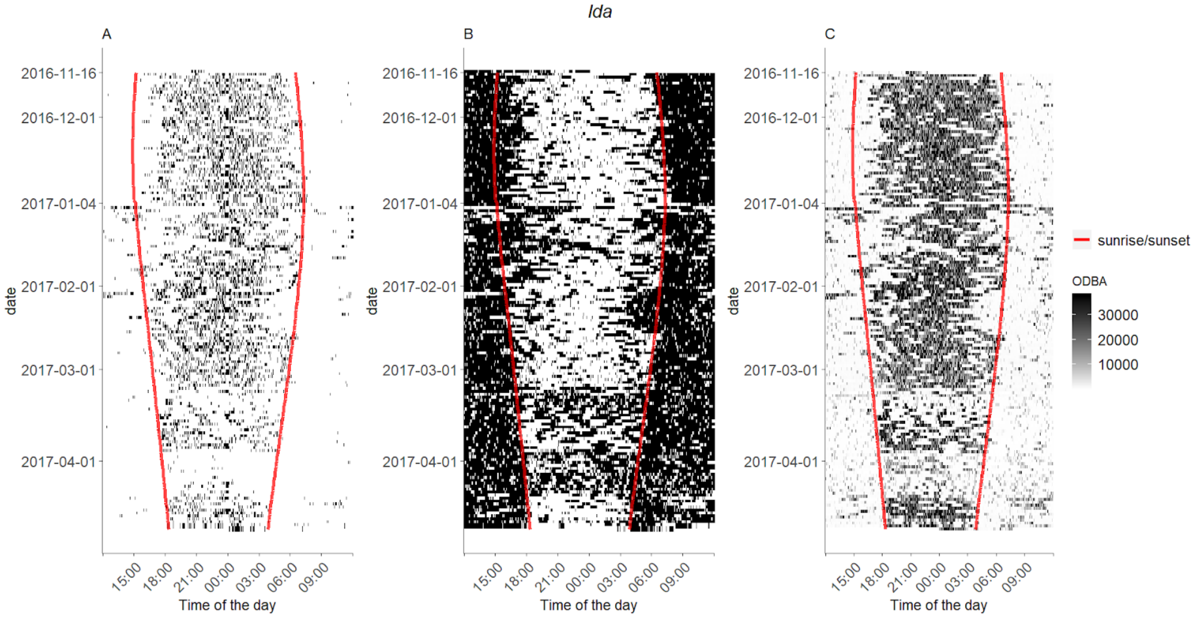

Figure S17: Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Ida". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.

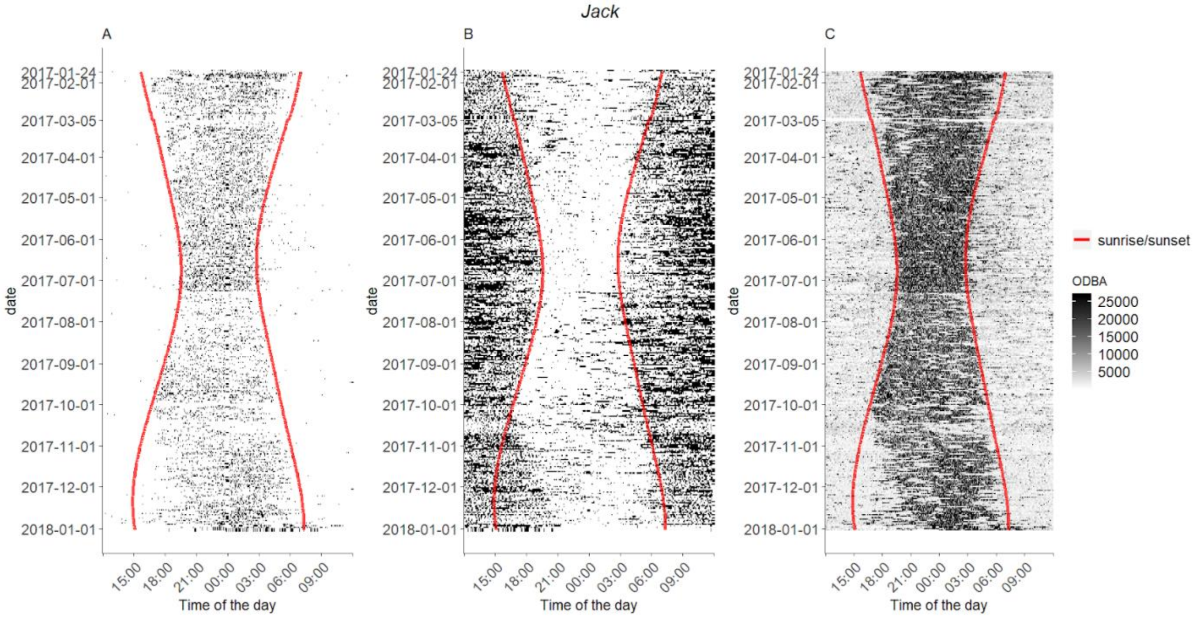

Figure S18: Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Jack". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.

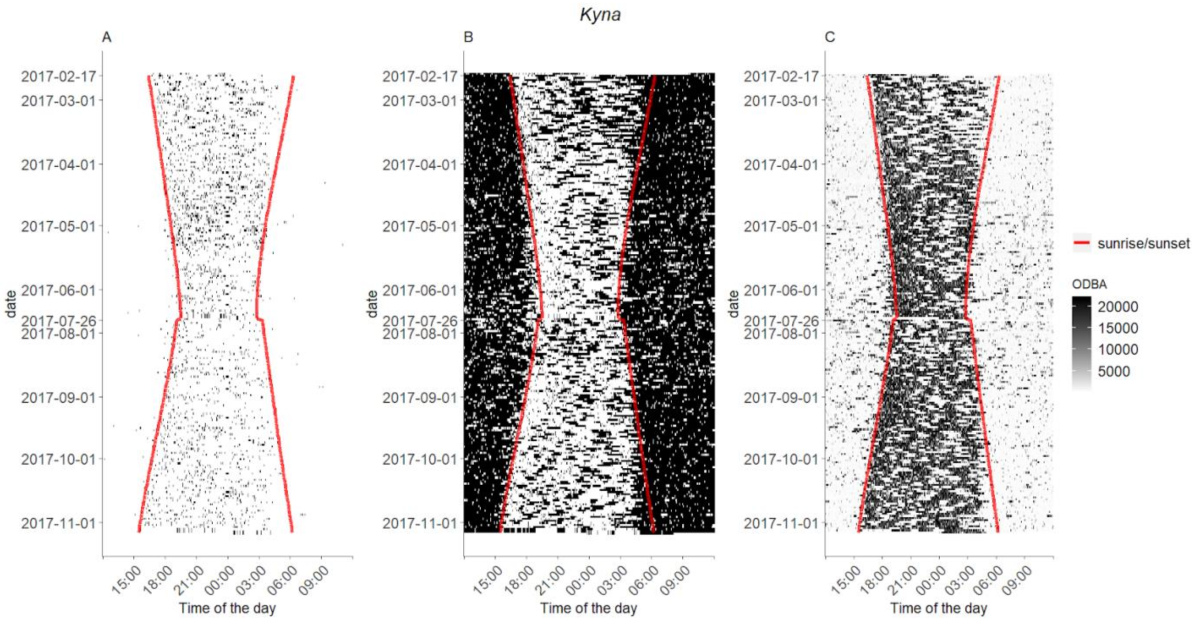

Figure S19: Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Kyna". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime. Discontinuities in the red line are caused by missing data due to the logger not recording data at the time.

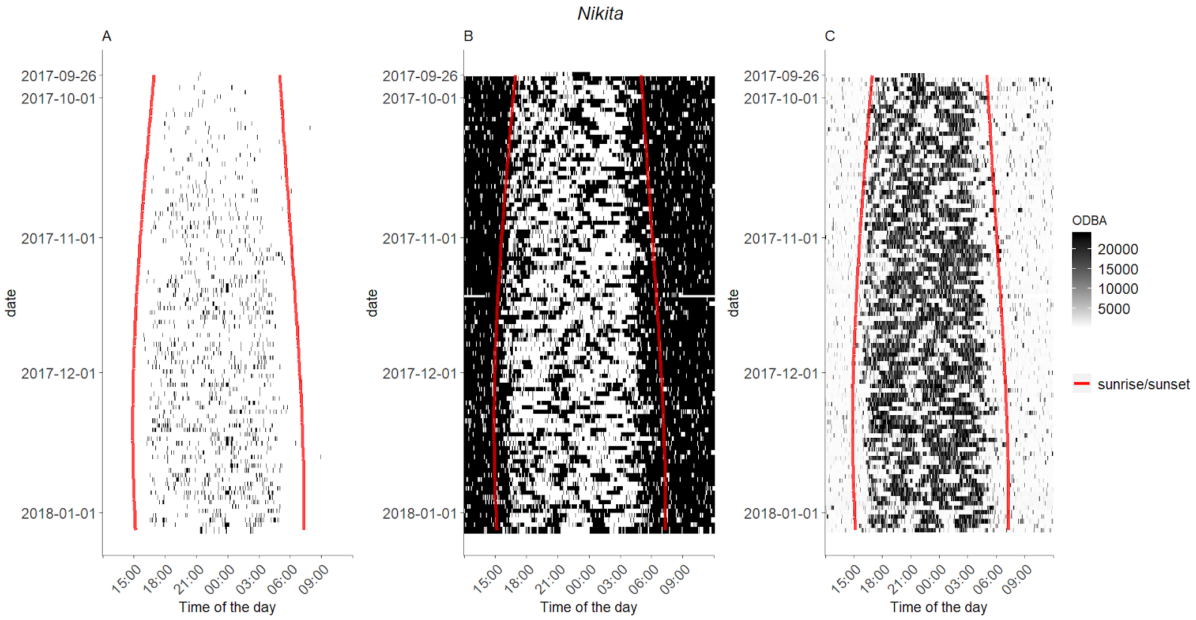

Figure S20: Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Nikita". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime.

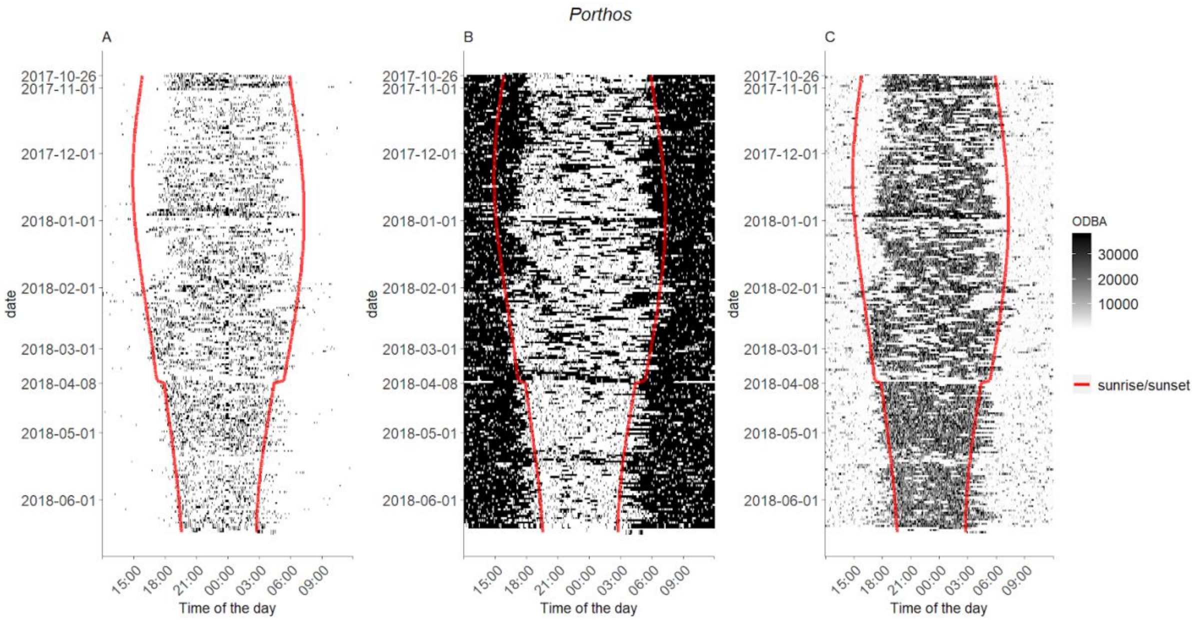

Figure S21: Temporal distribution of trotting (A), (B) resting and (C) ODBA values of "Porthos". The red lines indicate the sunset and sunrise. (A) Black spaces indicate times at which trotting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Trotting is predominantly predicted during the night. (B) Black spaces indicate times at which resting behaviour was predicted whereas white spaces indicate assignments of all other behaviours. Resting is predominantly predicted during the day. The switch between trotting, resting and other behaviours is mostly oriented at the sunset and sunrise. (C) Higher ODBA values are indicated by darker spaces. ODBA values are higher at night than during daytime. Discontinuities in the red line are caused by missing data due to the logger not recording data at that time.

Chapter 4: The perception of foxes in the general public

Elucidating the socio-demographics of wildlife tolerance using the example of the red fox (*Vulpes vulpes*) in Germany

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Author contribution

The original idea to socio-economic aspects of red fox perception was developed by Joachim Kimmerle (JK), Miriam Brandt (MB), Sophia E. Kimmig (SEK) and Ulrike Cress (UC). SEK elaborated the detailed concept. The questionnaire was developed by SEK and revised by Danny Flemming (DF). DF conducted data analysis (that were collected by the SOEP). SEK wrote the manuscript. DF, JK and MB contributed with substantial revisions to the manuscript.

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CONTRIBUTED PAPER

Elucidating the socio-demographics of wildlife tolerance using the example of the red fox (*Vulpes vulpes*) in Germany

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Abstract

As a consequence of increasing human-wildlife encounters, the associated potential for human-wildlife conflict rises. The dependency of conservation management actions on the acceptance or even the participation of people requires modern conservation strategies that take the human dimension of wildlife management into account. In the first place, conservationists therefore need to understand how people perceive wildlife. In the present study, we examined how wildlife perception varies with people's socio-demographic backgrounds in terms of age, gender, and education as well as the settlement structure of people's living environment and their general life satisfaction, using the red fox (*Vulpes vulpes*) as a model species. We used an interview-based survey of 2,646 participants, representative for the German population, for investigating their knowledge about, risk perception of, and attitude toward red foxes. We found a negative correlation between age and the risks perceived regarding foxes. Moreover, men held a more positive attitude and perceived less risk than women. Higher education was also associated with lower risk perception and a more positive attitude. The results further indicated that people who live in rural areas perceived higher risks regarding foxes and showed a less positive attitude than people in urban or suburban areas. Finally, people who perceived higher risks and held a less positive attitude supported lethal population management actions more often. However, we also found that perceived risks decreased with participants' general life satisfaction. Hence, wildlife perception is affected by various factors. Understanding the factors affecting wildlife perception is crucial for environmental communication and for fostering acceptance of conservation measures to improve conservation strategies.

KEYWORDS

attitude towards wildlife, human dimension, knowledge, perception of wildlife, risk assessment, wildlife management

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1 | INTRODUCTION

Almost all natural habitats worldwide are subject to human encroachment, which increases the level of shared human-wildlife living space and creates potential for human-wildlife conflict. At the same time, human-wildlife encounters also increase in decidedly industrialized regions, where human commensal species actively colonize urban habitats. Human-wildlife conflicts in such industrialized regions may result from wildlife damage and the fear of zoonotic diseases (Mackenstedt, Jenkins, & Romig, 2015). Generally, implications of human-wildlife coexistence may be social, economic, or health-related, and both actual and assumed implications may have a large impact on the acceptance of wildlife by the general public (Decker et al., 2012; Riley et al., 2002). Therefore, many conservationists propose that considering the social, physical, and economic well-being of people is central to a holistic conservation approach (Minteer & Miller, 2011).

Implementing this human dimension in conservation management may include approaches for loss or harm compensation (Naughton-Treves, Grossberg, & Treves, 2003; Nyhus, Osofsky, Ferraro, Madden, & Fischer, 2005), but also requires advocacy for conservation activities and of human-wildlife co-existence (Kansky, Kidd, & Knight, 2014). This is particularly true when people are forced to arrange themselves with the co-housing situation, for example, when they compete with threatened species and no alternative habitats are available for potential resettlements, or in urbanized areas, where species reach high densities (e.g., Prange, Gehrt, & Wiggers, 2004) and population reduction via hunting is not a feasible option.

Therefore, wildlife acceptance by humans is a crucial element of modern conservation and wildlife management (Enck et al., 2006; Greving & Kimmerle, 2020; Mascia et al., 2003). Ultimately, the preservation of nature is a human endeavor that requires getting stakeholders to support conservation aims (Salafsky, 2011). As a consequence, researchers, conservation agents, wildlife managers, and policy makers aim to raise awareness for the inherent value of biodiversity and to increase wildlife tolerance (Decker et al., 2016). Saunders, Brook, and Myers, (2006), thus, stated that we need to use psychology to save biodiversity (and human wellbeing). So, it is crucial to know which factors shape human perception of wildlife, that is, the knowledge about certain wildlife species, perception of the risks associated with these species, and the attitudes toward them.

Even though some research has been conducted in this field (Dickman, 2010), many studies on wildlife perception face methodological issues: When they are conducted as mail surveys, response rates are often low (e.g., Bjurlin & Cypher, 2005: 28%; or Thornton & Quinn, 2009: 29%). Surveys conducted among specific target groups, for example, members of conservation nonprofit organizations (NGOs), may achieve higher response rates but are not representative of the general public. Not being representative, for instance, might be a problem that most surveys face, since people with strong positive or negative opinions may be more likely to participate, leading to the underestimation of moderate positions. To study people's wildlife perceptions in relation to their socio-demographic backgrounds, we interviewed a large sample representative for the general public of Germany, with long-term data available for all participants. Since participants were interviewed personally, the response rate was virtually 100%. We chose the red fox (*Vulpes vulpes*) as an example of human-wildlife coexistence in Western Europe, a species abundant in urban as well as rural regions. In addition, humans and foxes have a long, ambiguous relationship. On the one hand, the mid-sized carnivore is described as beautiful, smart, and cute. Characterized as sly, yet charismatic, it appears in many folk tales that people grew up with. In recent years fox images have been used for decorative purposes on many daily life products, for example, as a print in fashion industry. On the other hand, foxes are associated with carrying infectious diseases and parasites, such as rabies and the fox tapeworm (*Echinoccocus multilocularis*), which may harm or even kill people (Combes et al., 2012). Although terrestrial rabies is extinct in Germany, many people still fear this disease, and echinococcosis is still present. Foxes are also potential predators of poultry and a threat to smaller companion animals and they cause damages in public and private gardens. Since attitudes toward foxes therefore vary from very negative to extremely positive, this species is well suited for assessing how these attitudes are influenced by people's backgrounds.

We related potentially relevant socio-demographic factors like age, gender, education, and place of residence to the outcome variables factual knowledge, risk perception, and attitude. Since a higher formal level of education usually goes along with greater general knowledge (Conway, Cohen, & Stanhope, 1991), which should lead to a more realistic estimation of risks, we hypothesized that a higher level of education would be associated with (a) higher levels of knowledge about foxes, (b) lower levels of risk perception, and (c) a more positive attitude toward foxes (Hypotheses 1a–c). It has been argued that due to urbanization and technification, people in cities have become alienated from nature and tend to romanticize wildlife (Heberlein & Ericsson, 2005). We therefore hypothesized that people who live in urban areas have (a) less knowledge, (b) lower levels of risk perception, and (c) a more positive attitude toward foxes than people who live in suburban and rural areas (Hypotheses 2a–c). People who have greater life satisfaction also have generally a more positive attitude toward other beings (Erdogan, Bauer, Truxillo, & Mansfield, 2012). Thus, we hypothesized that people with higher levels of general life satisfaction have more positive attitudes toward foxes (Hypothesis 3a) and perceive lower risks (3b). Finally, we investigated the relationship of the three measures, knowledge, risk perception, and attitude with people's opinions regarding fox-management strategies.

2 | MATERIALS AND METHODS

Participants were recruited via the Socio-Economic Panel (SOEP), located at the German Institute for Economic Research Berlin (DIW) that holds a highly reliable socio-demographic long-term dataset of the population in Germany. It is a private household-based longitudinal study that annually (re-) interviews up to 30,000 adult household members on numerous topics such as biography, employment, health, or satisfaction with political or personal circumstances. We used a slot in the 2016 survey of the *SOEP Innovation Sample* to conduct our study, with a representative subset of the SOEP sample (N = 2,646; age range: 18–95 years; mean age = 53.8 years; SD = 18.52; gender: N = 1,415 [53.5%] female; N = 1,231 [46.5%] male). The participants attended voluntarily and anonymously. Ethical approval was obtained from the Local Ethics Committee of the Leibniz-Institut fuer Wissensmedien. Due to the large sample size, all of the analyses were conducted using a 99% confidence level to minimize the risk of false-positive results.

Data were available on *age* (date of birth), *gender* (male/female), *education* (various classifications), *settlement structure* (rural/suburban/urban), and personal *life satisfaction* for all of the participants. According to their obtained school degree, *education* levels of participants were categorized as low (N = 894, 33.9%), medium (N = 839, 31.7%), or high (N = 710, 26.8%). Low education indicated that participants either had achieved no school leaving certificate or a degree from a secondary modern school from class level 5–9 (“Hauptschule”). A medium level of education meant that participants held a degree from a secondary modern school from class level 5 to 10 (“Realschule”), and high level of education meant that they got a university-entrance diploma from a secondary modern school from class level 5 to 12 or 13 (“Gymnasium” with degree “Abitur” or “Fachhochschulreife”). Two-hundred and three participants (7.7%) indicated having achieved a different certificate or did not provide information about their school-leaving qualifications. The participants' *settlement structure* was categorized pursuant to the criteria for spatial classification of the Federal Institute for Research on Building, Urban Affairs, and Spatial Development (BBSR) (Table A1, Appendix S1). The *life satisfaction* scale consisted of 10 items (Table A2) asking participants to rate their satisfaction with different aspects of their lives from 0 (not satisfied) to 10 (very satisfied). Internal consistency (Cronbach's alpha) of this scale was $\alpha = 0.69$. All ratings were summed up and divided by the number of items.

We designed a questionnaire that inquired into people's knowledge about, risk perception regarding, and attitude toward foxes. Earlier versions of the questionnaire were tested in a laboratory setting with a smaller sample before being implemented in the survey presented here (Flemming, Cress, Kimmig, Brandt, & Kimmerle, 2018). In order to measure people's factual *knowledge*, we used 11 statements about foxes—with six statements being correct and five statements being wrong (Table A3). Participants were asked to classify the statements as true or false, and each correct assessment was coded with one point. All points were added, resulting in a scale from 0 to 11 points, with higher values indicating greater knowledge.

The *risk perception* questionnaire consisted of five items, with each item representing an infectious disease. Participants had to indicate for each disease how they perceived the risk of infection for themselves and for domestic animals on a 5-point Likert scale from 0 (no risk) to 4 (very high risk). They could also choose the option “not able to say” (this choice was treated as missing data in the analysis and replaced by the mean score of all participants on that item). The diseases listed were rabies (a viral disease that causes inflammation of the brain in humans and other mammals), echinococcosis (a parasitic disease caused by the fox tape worm. In the questionnaire referenced as “fox tape worm” because the expression echinococcosis is less known), distemper (a viral disease that affects domestic dogs and wild animals), mange (a skin disease caused by parasitic mites) and foot-and-mouth disease (FMD, a viral disease that affects cloven-hoofed animals). Of these, only echinococcosis, can actually be transmitted from foxes to humans in Germany (the country having been declared free of terrestrial rabies by the World Health Organization [WHO] in 2008) while mange and distemper can be transmitted to companion animals. We also included Morbus metum, which is a fictitious disease that was not included in the further analyses, since less than 50% of the participants provided an answer to this item. The sum total was divided by the number of items, resulting in a risk perception score between 0 and 4, with higher values indicating perception of higher risks. Internal consistency (Cronbach's alpha) of this scale was $\alpha = 0.89$.

The *attitude* questionnaire consisted of six items, such as "I consider foxes in urban environments a pest," that participants had to rate on 7-point Likert scales from 1 (completely disagree) to 7 (fully agree). Items 2, 5, and 6 were inverted and recoded before summing up all the items (Table A4). The score was divided by the number of items, resulting in an attitude score between 1 and 7, with higher values indicating a more positive attitude. Internal consistency (Cronbach's alpha) of this scale was $\alpha = 0.70$.

We also asked participants how wild fox populations should be managed. They could opt for protecting and supporting foxes, for area-wide hunting, or for not intervening. They also had the option "no opinion on fox management". Answers were coded with 0 for hunting, 1 for neutrality, and 2 for a supporting position. No opinion was coded as a missing value.

We present descriptive statistics to provide frequency information on the sample's education level, settlement structure, and fox population management preferences. We also provide mean scores and standard deviations for life satisfaction, knowledge about foxes, risk perception, and attitude. Moreover, we conducted correlation analyses with the variables knowledge, risk perception, and attitude. To examine the role of age and gender, we conducted a multivariate analysis of variance with gender as a fixed factor and age as a covariate. In order to test the hypotheses, we conducted multivariate analyses of variance and regression analyses. All statistical analyses were conducted using SPSS Statistics 25.

3 | RESULTS

The participants' knowledge about foxes was on average $M = 4.77$ ($SD = 1.54$) out of 11 possible points. Their average risk perception was $M = 1.40$ ($SD = 0.93$) on a scale from 0 to 4 (Figure 1). Their mean attitude toward foxes was $M = 4.27$ ($SD = 1.47$) on a scale from 1 to 7. Regarding the fox population management 30.8% of the participants indicated a supporting position, 9.6% preferred hunting, 40.6% opted for not intervening, and 19.1% had no opinion on fox management (Figure 2a). A total of 38.6% of the participants lived in urban, 31.6% in suburban, and 29.9% in rural regions. Their average life satisfaction was $M = 6.70$ ($SD = 1.54$) on a 10-point scale.

We found a positive correlation between factual knowledge and attitude ($r = .118$, $p < .001$) and a negative correlation between factual knowledge and risk perception ($r = -.062$, $p = .01$): The more participants knew about foxes, the more positive their attitude was toward them and the less they perceived them as a risk. There was a negative correlation between attitude and risk perception ($r = -.185$, $p < .001$), such that the lower the risk people perceived, the more positive their attitude was toward foxes.

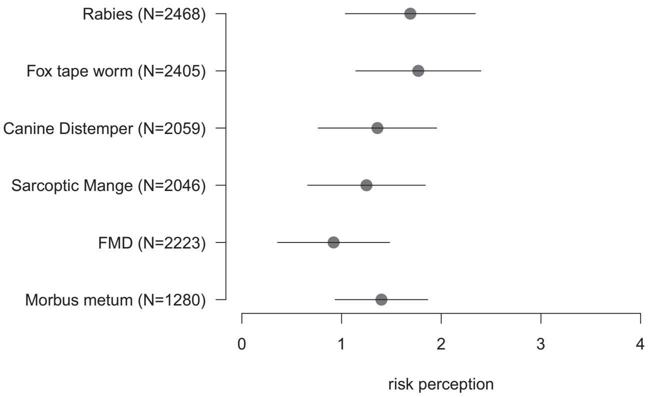

FIGURE 1 Means and *SD*s of the participants' risk perception (from 0 (no risk) to 4 (very high risk)) regarding the following diseases: rabies, echinococcosis ("fox tape worm"), canine distemper, sarcoptic mange, foot-and-mouth disease (FMD and morbus metum. Of the diseases listed, only the fox tapeworm is a threat to people in Germany. Terrestrial rabies was eradicated in Germany in 2008, so it cannot be contracted by foxes anymore. Canine distemper and mange can be transmitted from foxes to companion animals. Morbus metum is a fictitious disease

A multivariate covariance analysis controlling for age revealed significant differences between men and women, $F(3, 2{,}641) = 8.228$, $p < .001$; Wilk's $\Lambda = 0.991$, partial $\eta^2 = 0.009$ (Table A5). Women and men did not differ in knowledge, $F(2, 2{,}644) = 0.186$, $p = .666$, but differed significantly in risk perception, $F(2, 2{,}644) = 6.888$, $p = .009$; partial $\eta^2 = 0.003$, and attitude, $F(2, 2{,}644) = 21.375$, $p < .001$; partial $\eta^2 = 0.008$): Men perceived significantly lower risk ($M = 1.35$, $SD = 0.91$) than women ($M = 1.44$, $SD = 0.94$). Men also held a more positive attitude toward foxes ($M = 4.41$, $SD = 1.42$) than women ($M = 4.15$, $SD = 1.50$). The covariate age affected neither knowledge, $F(1, 2{,}645) = 0.934$, $p = .334$ nor risk perception, $F(1, 2{,}645) = 0.249$, $p = .618$), but had a significant effect on attitude, $F(1, 2{,}645) = 41.175$, $p < .001$; partial $\eta^2 = 0.015$. The younger the participants were, the more positive were their attitudes toward foxes.

In order to test Hypotheses 1a–c, we conducted a multivariate analysis of variance that revealed an overall significant impact of education, $F(6, 4{,}820) = 18.829$, $p < .001$, Wilk's $\Lambda = 0.955$, partial $\eta^2 = 0.023$. However, participants with different educational levels did not differ in their knowledge about foxes, rejecting Hypothesis 1a, $F(2, 2{,}412) = 0.051$, $p = .950$. Hypotheses 1b and c, in contrast, were supported: Depending on their educational levels, participants differed in their risk perception, $F(2, 2{,}412) = 24.155$, $p < .001$, partial $\eta^2 = 0.020$ as well as in their attitude toward foxes,

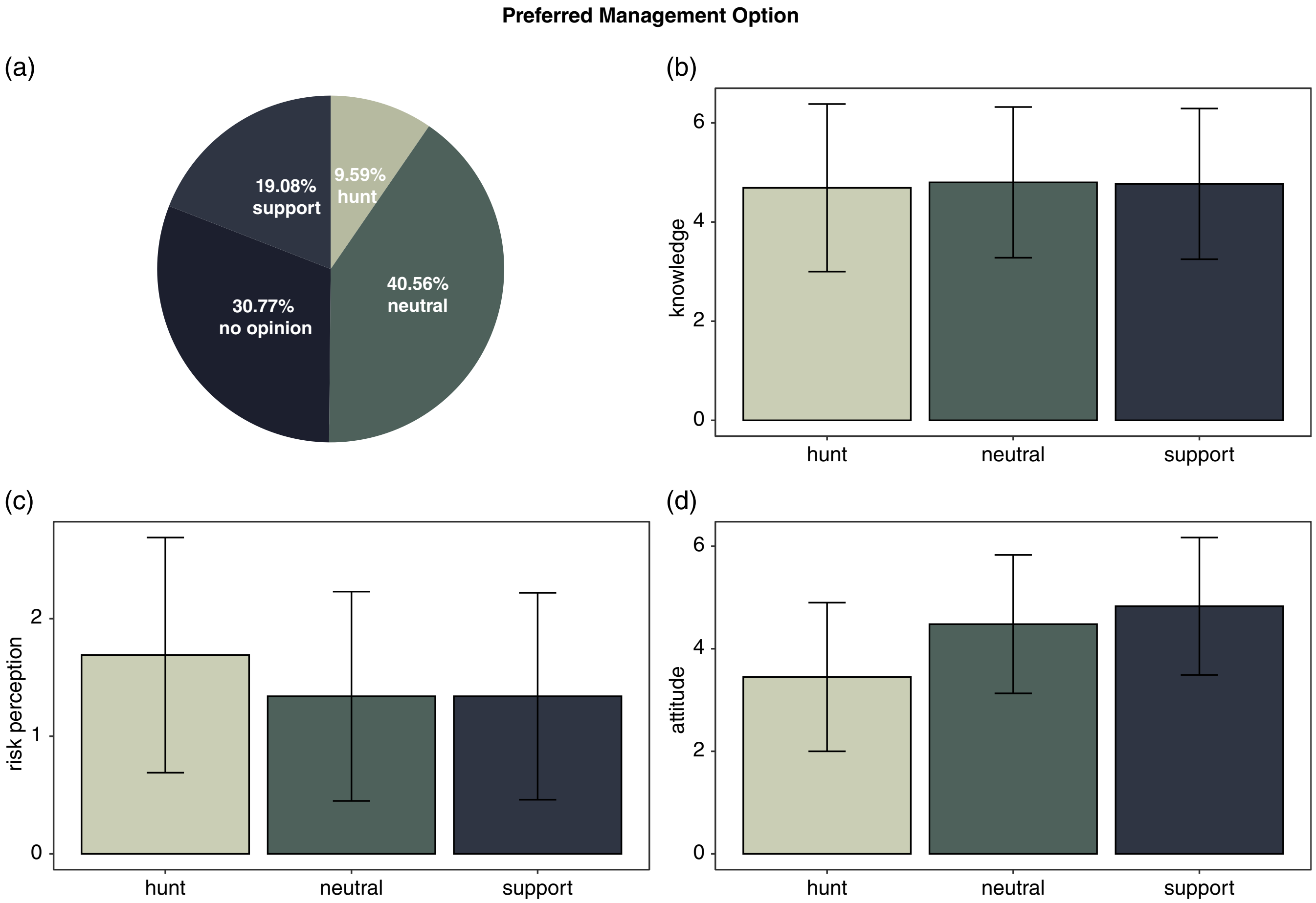

FIGURE 2 Preferred actions regarding fox management strategies in Germany (2a) with the following options: "hunt," "do not intervene (neutral)," "protect and support," and "no opinion on management," as well as means and standard errors for knowledge (2b), risk perception (2c), and attitude (2d) depending on participants' preferred fox management strategies

TABLE 1 Knowledge, risk perception, and attitude of participants in relation to their educational level (low, medium, or high) and settlement structure (urban, suburban, or rural)

	Educational level						**Settlement structure**					
	Low		**Medium**		**High**		**Urban**		**Suburban**		**Rural**	
	Mean	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***
Knowledge	4.79	1.54	4.77	1.55	4.77	1.53	4.79	1.49	4.72	1.64	4.66	1.54
Risk perception	1.55	1.00	1.40	0.91	1.22	0.82	1.34	0.87	1.39	0.94	1.57	0.96
Attitude	3.98	1.47	4.24	1.39	4.63	1.43	4.34	1.52	4.03	1.35	4.07	1.48

Note: Knowledge: scale range from 0 to 11; risk perception: scale range from 0 to 4; attitude: scale range from 1 to 7.

$F(2, 2{,}412) = 40.518$, $p < .001$, partial $\eta^2 = 0.033$. Post hoc tests with Bonferroni corrections revealed that all of the groups (low, medium, and high educational levels) differed significantly from each other regarding both risk perception and attitude. Higher education levels were associated with more positive attitudes and lower risk perception, and lower education was associated with more negative attitudes and higher risk perception (Table 1).

For testing Hypotheses 2a–c, we conducted a multivariate analysis of variance that revealed an overall significant impact of settlement structure, $F(6, 2{,}884) = 4.897$, $p < .001$; Wilk's $\Lambda = 0.980$, partial $\eta^2 = 0.010$. While knowledge did not vary with settlement structure, $F(2, 1{,}444) = 0.915$, $p = .401$, the data supported our hypotheses regarding risk perception, $F(2, 1{,}444) = 8.553$, $p < .001$, partial $\eta^2 = 0.012$, and attitude, $F(2, 1{,}444) = 7.132$, $p < .001$, partial $\eta^2 = 0.010$).

TABLE 2 Preferred management strategies in relation to knowledge, risk perception, and attitude

	Hunt		Neutral		Support	
	Mean	***SD***	**Mean**	***SD***	**Mean**	***SD***
Knowledge	4.69	1.69	4.80	1.52	4.77	1.52
Risk perception	1.69	1.00	1.34	0.89	1.34	0.88
Attitude	3.45	1.45	4.48	1.35	4.83	1.34

Post hoc tests with Bonferroni corrections revealed that people who live in rural areas had a significantly higher risk perception than people who live in urban ($p < .001$) or suburban ($p = .010$) areas. People who live in urban areas also had a more positive attitude toward foxes than people who live in suburban ($p = .002$) or rural areas ($p = .010$) (Table 1).

We hypothesized that life satisfaction also influences attitude toward foxes (Hypothesis 3a) and risk perception (Hypotheses 3b). There was indeed a slightly positive regression coefficient for attitude, but it was not significant on the 1% level (standardized $\beta = 0.044$; $p = .024$; $R^2 = 0.044$). We found, however, that the more satisfied people were with their lives, the lower their risk perception was (standardized $\beta = -0.012$; $p < .001$; $R^2 = 0.12$).

Finally, we compared people who held different views on management options with regard to their knowledge, attitude, and risk perception, and found significant differences, $F(6, 2{,}141) = 35.342$, $p < .001$; Wilk's $\Lambda = 0.061$, partial $\eta^2 = 0.939$. People who had different views on management options did not differ with regard to knowledge, $F(2, 2{,}141) = 0.478$, $p = .620$, but with regard to attitude, $F(2, 2{,}141) = 100.602$, $p < .001$, partial $\eta^2 = 0.086$, and risk perception, $F(2, 2{,}141) = 16.975$, $p < .001$, partial $\eta^2 = .016$ (Table 2). Post hoc tests showed that all groups differed significantly in their attitude: Participants who were in favor of supporting fox populations had a more positive attitude toward foxes than those who preferred not to manage them at all (neutral). Supportive and neutral participants both had a more positive attitude than those who preferred hunting. Participants also significantly differed in risk perception: People who favored hunting perceived higher risks than those who were neutral or supportive. People who were neutral did not differ in risk perception from those who wanted to support fox populations (Figure 2b–d).

4 | DISCUSSION

In recent years, many researchers have tried to account for the human dimension of wildlife management and conservation (Miller, Minteer, & Malan, 2011). One theory on human responses to wildlife argues that the number of people who value conservation may decline if costs or perceived risks of coexisting with wildlife increase disproportionately to the benefits (Decker et al., 2012). The results of our study show the importance of factoring in sociodemographic background when trying to gauge people's perception of wildlife.

With less than half of the possible points achieved on average, the participants' factual knowledge about foxes in this study was rather low. This is probably not due to people not being familiar with foxes, as Hooykaas et al. (2019) showed that in the Netherlands, the red fox was correctly identified by 97.2% of primary school children and 99.2% of participants from the general public. Thus, omnipresence of a species apparently does not necessarily lead to an increase in factual knowledge about it. Risk perception (mean 1.4 out of 4) was lower than randomly expected and the average attitude toward foxes was rather positive (mean 4.27 out of 7). In line with these results, a study on perception of foxes in Munich, Germany found that the majority of inhabitants were “pleased to see a fox in the community and felt the animals have a right to live” (König, 2008, p. 101).

While factual knowledge about foxes was equivalent among gender and age groups, some differences in the perception of risks and in attitude became apparent. In line with other studies, women perceived more risks from foxes and, in contrast to those studies, also held a less positive attitude toward them (Thornton & Quinn, 2009; Zinn & Pierce, 2002). König (2008) found that people are more afraid of fox-borne diseases when they have children in their households. One could thus assume that health- and safety-related concerns are more important to women who are still more often responsible for raising children in our society (see also Dietz, Kalof, & Stern, 2002). Some studies suggest that women generally tend to report stronger environmental attitudes and concerns than men (Luchs & Mooradian, 2012; Scannell & Gifford, 2013; Tikka, Kuitunen, & Tynys, 2000). However, risk perception is complex and other findings suggest that socio-political factors, such as power and status, are also strong determinants of people's perception of risks (e.g., Flynn, Slovic, & Mertz, 1994).

In the United States, adolescent conservation behaviors have shown a downward trend since the 1970s (Wray-Lake, Flanagan, & Osgood, 2010, see also Thornton & Quinn, 2009). This seems to contrast with our finding that younger people held a more positive attitude toward foxes than older people. However, in a review on pro-environmental concerns and behavior by Gifford and Nilsson (2014) the authors conclude that those two measures do not converge well: Several studies have shown that older people report engaging in more pro-environmental behavior than younger people (e.g., Pinto, Nique, Añaña, & Herter, 2011; Swami, Chamorro-Premuzic, Snelgar, & Furnham, 2011). But research also shows that younger people report being more concerned about the general environment than older people (e.g., Arcury & Christianson, 1993; Klineberg, McKeever, & Rothenbach, 1998). Generally, it is difficult to detect whether apparent age effects are caused by aging itself or may be the result of a cohort effect (e.g., due to a specific experience in a generation) or an era effect (e.g., due to a general trend in society).

We found no effect of settlement structure on fox-related knowledge. However, people living in urban agglomerations showed a more positive attitude toward foxes than people in suburban or rural areas, while people living in rural areas perceived higher risks from foxes than people in urban and suburban areas. This has also been shown for other carnivores, except coyotes (Kansky et al., 2014; Williams, Ericsson, & Heberlein, 2002). According to Manfredo (2008), these differences could be explained by the fact that urban residents are less impacted in their livelihoods by wildlife: On the protection-use scale (Fulton, Manfredo, & Lipscomb, 1996; Manfredo, Teel, & Bright, 2003), wildlife perception is ranging from a utilitarian end with the belief that wildlife should be managed and used for human benefit to a protection end, at which people think wildlife has an intrinsic value and should have rights similar to those of humans. In this context, urbanization and modernization may lead urban residents to be more tolerant of wildlife, because they view wildlife as beings with rights rather than as a food source. This is consistent with literature showing less anthropocentric tendencies in urban residents (e.g., Huddart-Kennedy, Beckley, McFarlane, & Nadeau, 2009) and describing an association between urbanization and increased concern about animal welfare (e.g., Hays, 1987; Mertig, Dunlap, & Morrison, 2002).

We found an effect of educational level on attitude and risk perception but, not on fox-related knowledge. Higher education levels were associated with lower levels of risk perception and a more positive attitude. These findings correspond well with prior research (e.g., Hanisch-Kirkbride, Riley, & Gore, 2013), as well as with the observation that utilitarian wildlife values are associated with lower levels of education and that education shifts these values toward an appreciation of wildlife (Inglehart & Baker, 2000; Manfredo et al., 2003). More generally, they are also in line with findings from Karanci, Aksit, and Dirik (2005) who showed that higher educated individuals tend to worry less about possible future risks but show a higher sense of control and preparedness.

We found that the more satisfied people were with their lives, the lower their risk perception was. This could perhaps be explained by the fact that people with lower levels of emotional stability worry about many aspects of life, among them about environmental issues (Hirsh, 2010). Wildlife perception seems to be multifactorial (McShane et al., 2011) and several personality or self-construal related factors, such as openness, agreeableness, or conscientiousness have been shown to be linked to environmental engagement (Milfont & Sibley, 2012). However, life satisfaction depends on a person's living circumstances and can be influenced at least to some extent by local management actions. Thus, we may need to include considerations of life satisfaction in conservation management attempts. Social and economic interventions around protected areas in Nepal, for example, led to more favorable attitudes toward conservation (Baral & Heinen, 2007). For some local areas, conflicts, or stakeholder groups, it may therefore be promising to invest in development of infrastructure or other economically or culturally relevant areas, rather than in information campaigning only, to improve the people's quality of life in general.

Finally, we found that a majority of the participants (who were representative for the German population) preferred either not to intervene in the fox population or did not have an opinion on fox management. Only a small proportion was in favor of lethal population control (less than 10%). This corresponds with König's (2008) prediction who stated on the basis of his local study that "it is to be expected that radical solutions such as killing the foxes are unlikely to be accepted among the population" (p. 101).

We also found that there was a relationship among attitudes, perceived risks, and preferred management strategies. Generally, the perception of higher risks of getting infected with fox-borne diseases was correlated with a more negative attitude. It is important to note that only three of the diseases listed pose an actual threat to humans or domestic animals, but that more than those three were seen as a threat by the participants, indicating that the true risk level may be quite irrelevant in determining perceived risks (see also Figure 1). This corresponds to the findings of Decker et al. (2012) that perceived risks of wildlife associated diseases are a growing concern. These perceived risks influenced people's attitudes and their opinion on management options: People with positive attitudes and low-risk perception preferred neutral coexistence with or active support of fox populations, while people with higher risk perceptions and more negative attitudes were in favor of hunting.

It seems plausible that better knowledge about foxes would prevent an overestimation of risks that are based on false assumptions. This would, consecutively, result in a more positive attitude and finally in increased tolerance for human coexistence with foxes. We thus expected species-related knowledge to influence wildlife perception. Maybe another set of questions on factual knowledge would have shown a different picture, yet we intended to test the general effect of species-specific knowledge and therefore not only included questions that directly relate to possible threats.

There was an indirect effect of factual knowledge on management preferences, that is, we could find a positive correlation between fox-related knowledge and attitude and a negative one between knowledge and risk perception, which in turn show significant impact on management attitude. Interestingly, however, fox-specific knowledge did not significantly affect the participants' management preferences and was not influenced by the socio-demographic factors tested. One possible explanation is that increased knowledge of wildlife might simply be used to support pre-existing opinions (Bjurlin & Cypher, 2005; Kellert, Black, Rush, & Bath, 1996). This could indicate that more knowledge about fox-related diseases, for example, just increases already present fears, an effect that König (2008) reported regarding the small fox tapeworm (*Echinococcus multilocularis*). In a mail survey in Boulder, the United States, Hunter and Rinner (2004) also found that people with more eco-centric perspectives placed greater priority on species preservation, regardless of species-related knowledge. Accordingly, successful conservation management should not only provide species-specific knowledge but also advocate the significance of ecological integrity and biological diversity more broadly (Hunter & Rinner, 2004).

5 | CONCLUSION

Obtaining people's support is crucial for conservation success. However, as we have shown here, the perception of wildlife is influenced by a complex interplay of factors (see also McShane et al., 2011). Determining how to best promote conservation actions is challenging when wildlife tolerance differs among socio-demographic groups. Since the general level of education and even life satisfaction have an impact on the perception of wildlife, studying the socio-demographics behind wildlife tolerance may not be sufficient. We would need to additionally investigate how to reach out to different target groups and study how different communication styles and formats influence wildlife perception of those groups.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

M.B., S.E.K., J.K., and U.C. designed the research. D.F., J.K., and S.E.K. conducted data analysis and plotting. S.E.K., and D.F. wrote the manuscript. All authors contributed to the development of the questionnaire, contributed to the drafts and gave final approval for publication.

ETHICS STATEMENT

All research was carried out in accordance with standard research practices.

DATA AVAILABILITY STATEMENT

The data used in this study contain sensitive personal information. Due to German data protection regulations they are therefore not publicly available for download but will be made available upon request by the Socio-Economic Panel (SOEP) Research Data Centre: https://www.diw.de/en/diw_02.c.222829.en/access_and_ordering.html#242008.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Appendix

Table A1. Spatial classification of the Federal Institute for Research on Building, Urban Affairs and Spatial Development (BBSR)

Type of region	Classification
Urban regions	Regions in which at least 50% of the population lives in large and medium-sized cities and in which there is a large city with 500,000 inhabitants or more. Additionally, regions with a population density of at least 300 inhabitants/km² excluding large cities.
Regions with beginning urbanization	Regions in which at least 33% of the population lives in large and medium-sized cities with a population density of 150 to 300 inhabitants/km² and regions in which at least one large city is located and which have a population density of at least 100 inhabitants/km² excluding large cities.
Rural regions	Regions in which less than 33% of the population lives in large and medium-sized cities, with a population density below 150 inhabitants/km².

Table A2. Satisfaction with different aspects of personal life according to SOEP core data set V33.

SOEP Variable	Aspect of life
pzuf 01	Health
pzuf 02	Work
pzuf 03	Household work
pzuf 04	Household income
pzuf 05	Personal income
pzuf 07	House/Flat/Housing
pzuf 08	Leisure time
pzuf 13	Family
pzuf 14	Friends
pzuf 20	Sleep

Table A3. Factual knowledge: 11 statements about foxes that participants were asked to classify as true or false.

Statement	**Correct Answer**
Foxes are able to climb trees	True
There is a risk of getting infected with rabies through wild foxes in Germany	False
Foxes are socially flexible and live in couples as well as in small groups	True
Foxes in the wild have lifespans of up to 20 years	False
In Germany, infections with the fox tape worm are extremely rare	True
A fox has approximately half the body weight of a shepherd dog	False
Approximately half a million foxes are shot each year in Germany	True
Due to fear of humans, foxes in cities flee earlier when being approached	False
In foxes, both parents participate in parental care	True
In Germany around 5 to 10 people per year get infected with the fox born disease Morbus metum	False
Amongst other things, foxes consume insects, earthworms and fruit	True

Table A4. Questionnaire for measurement of attitude toward foxes, containing six statements to be rated on seven-point Likert scales.

Please state to what extent, in your opinion, the following statements are true.

	1 Completely disagree	2	3	4	5	6	7 Fully agree
I would be pleased having a fox in my garden/living environment	☐	☐	☐	☐	☐	☐	☐
Foxes are dangerous for children	☐	☐	☐	☐	☐	☐	☐
I enjoy seeing foxes	☐	☐	☐	☐	☐	☐	☐
Foxes are part of nature. They belong to our environment and should be accepted around humans.	☐	☐	☐	☐	☐	☐	☐
I consider foxes in urban environments a pest.	☐	☐	☐	☐	☐	☐	☐
Foxes are potential carriers of diseases and shouldn’t be around people.	☐	☐	☐	☐	☐	☐	☐

Table A5. Knowledge, attitude and risk perception of men and women.

	Men		**Women**	
	Mean	SD	Mean	SD
Knowledge	4.78	1.54	4.76	1.54
Attitude	4.41	1.42	4.15	1.50
Risk perception	1.35	0.91	1.44	0.94

General discussion

Numerous studies have shown that living in urban environments affects both non-adaptive and adaptive evolution of wildlife (Johnson & Munshi-South, 2017). Many urban dwelling species have not specifically evolved new or modified traits as evolutionary adaptations to the urban environment (so far), rather their inherent behavioural flexibility (or phenotypic plasticity) allows for an adjustment to the urban habitat (Lowry et al., 2013). The red fox is described as such a flexible species (e.g. Baker et al., 2000; Lucherini & Lovari, 1996; Macdonald, 1983) with a high capacity to adjust to novel habitats. Our study shows that red foxes do dwell in metropolitan areas and move in and disperse through the urban matrix. Nevertheless our study also shows that foxes are constrained in their movements by human activities, with interesting and far-ranging consequences on both the individual and the population level.

Navigating the urban landscape

The urban habitat is characterised by a high degree of artificiality, from sealed surfaces to highly built-up areas that may hinder animal movements due to (extreme) habitat fragmentation (e.g. Grimm et al., 2008; Holderegger & Di Giulio, 2010; Seto et al., 2011). We therefore studied the effect of physical properties of the landscape on red fox dispersal and genetic structure at the population level, as well as their spatial behaviour on an individual scale. Our results showed that physical barriers may affect red fox movement abilities in a minor way. For instance, larger waterbodies, streams and channels limited gene flow through the urban matrix, but their “resistance” to gene flow was low, especially when compared to the sevenfold higher resistance values of the administrative Berlin city border. This is in line with reports that red foxes frequently cross rivers (Adkins & Stott, 1998). Also, densely built-up areas had just a slight negative effect on gene flow and were also not avoided by red foxes when considering their individual-based habitat selection. The population genetic results suggest that there is some genetic substructure between the eastern and the western part of the city fox population, although the historic division of Berlin because of the Berlin wall makes it difficult to disentangle the effects of current and past barriers.

Urban landscape features often act as barriers to gene flow (Storfer et al., 2010) but a review on urban ecological genetic studies found no evidence of consistently increased between-population genetic differentiation associated with urbanisation (Miles et al., 2019). Past research on the genetic structure of red fox populations between rural and urban areas only found population differentiation as a consequence of a founder effect that had already dissolved (DeCandida et al., 2019; Gloor, 2002). Even a lack of geographical structuring has been described for the red fox (Teacher et al., 2011). We detected two distinct genetic fox populations, roughly corresponding to the city and the rural area outside the city boundaries respectively (with precise boundaries depending on the methodological approach). Although physical landscape elements may affect red fox movement probabilities this did not seem to be the driving factor for dispersal impediment and genetic differentiation (see also below). Red foxes even made use of man-made structures by choosing motorways and railways as dispersal corridors. This is in line with former findings of red foxes using linear transport infrastructure as links in the urban matrix (Kolb 1984) and might serve increased travel speeds. High resolution step selection analysis in wolf (*Canis lupus*), woodland caribou (*Rangifer tarandus caribou*), moose (*Alces alces*) and black bear (*Ursus americanus*) revealed that all species moved faster while on anthropogenic linear features (Dickie et al., 2020).

According to our results, neither dispersal movements nor daily space use of red foxes seemed to depend on natural-like patches within the city habitat such as natural habitat remnants or urban forests. In the urban context such natural areas are minimally modified for human use, compared to built-up, developed areas that have been substantially altered for residential, recreational, commercial or industrial purposes (Fischer et al., 2015). They therefore often serve as refuge areas in urban environments and are of key importance to urban biodiversity conservation (Rudd et al., 2002; Aronson et al., 2017). For red foxes, however, urban green spaces seemed to play a minor role at the population level and were even actively avoided at the individual level. The conductance of such green spaces to gene flow was low and according to the habitat selection models analysed at the individual level, red foxes avoided public parks. They even more strongly avoided urban forests which are probably the least altered patches in the Berlin city area.

In contrast, wasteland (including industrial remains and verges along railways) was strongly preferred by red foxes, followed by gardens of residential houses. The latter may not only provide cover but also represent important food sources. Gardens offer many possible food items for red foxes, including fruits, food for companion animals and small rodents [Baker and Harris, 2007]) and the abundance of food is much higher than in forested areas (Hansen et al., 2020). However, the availability of food is also high in public parks, providing prey such as mice, voles and earthworms as well as human leftovers in considerable amounts, especially in central city areas. Nevertheless, these parks are avoided by red foxes. Food availability cannot adequately explain the strong preference for wasteland either. Thus, other factors such as human induced disturbance and human activities are essential for an understanding of dispersal as well as space use patterns in urban red foxes, particularly when considering the risks involved when using transport infrastructure as movement corridors or verges along railways for shelter or breeding.

Behavioural adjustments…

Urban wildlife often displays behaviours that differ from those of their rural counterparts (Lowry et al., 2013). This pattern is reflected by the strong limiting effect of the Berlin city border, compared to the slight impediments caused by physical barriers such as waterbodies, on possible immigration by rural red foxes, whereas urban red foxes routinely make use of man-made structures. Our landscape genetics results suggest that urban red foxes are accustomed to the city to a certain degree whereas their rural conspecifics face behavioural barriers to cross the city border and disperse to the urban habitat. According to Lowe et al. (2013) some individuals or species might exhibit behavioural traits (or a particular temperament) that are inherently well suited to occupying urban habitats, such as a high level of tolerance to disturbance (Lowry et al., 2013) and the foxes definitely showed a certain degree of tolerance towards human presence. However, our results suggest that even city foxes avoid human-dominated areas when possible.

Other urban ecological studies concluded that food dispersion cannot sufficiently explain red fox distribution and space use (e.g., Cavallini and Lovari, 1994). Our results suggest that human presence and activity may be key drivers for the selection of dispersal routes and habitat choices. For instance, as mentioned above, the habitat selection analyses we conducted showed that foxes exhibited avoidance behaviour towards urban forests and public parks, although especially the latter provides both cover and food. This is also true for allotment gardens that are likewise avoided. All these areas are characterised by a rather natural habitat like structure (compared to built-up areas, streets or places) but also by high levels of human presence (and to some extent of their companion animals). In a camera trap study on habitat use in red foxes, landscape, food availability and human disturbance influenced red fox appearance. In forest-dominated areas, human activities had a negative effect whereas in proximity to urban areas the main driver determining fox presence was food availability (Alexandre et al., 2020). Camera trap studies can be of limited use for the study of habitat selection, since in a study on red fox habitat selection in a rural area, camera trapping supported a high level of red fox activity in open land, whereas GPS telemetry revealed the opposite (Fiderer et al., 2019). Our results therefore provide a thorough insight into the importance of human presence for red fox habitat use. Red foxes specifically selected wasteland areas inaccessible to humans, irrespectively of their structural composition or degree of artificiality (regarding imperviousness or buildings). Human presence is also much lower (and much more predictable) in private residential gardens that were preferred by the foxes compared to public green spaces. To assess the impact of human presence beyond the issue of accessibility of urban sites to humans, we also included population density into our spatial analysis. When the areas used by red foxes were compared to the available habitat, increasing human population density clearly was avoided and only when human population density had been taken into account, imperviousness was avoided too. This avoidance of high human population densities was particularly distinct during daytime, at times of high human activity.

The results suggest not only a spatial but also a temporal adjustment to human presence. Such temporal activity patterns are mechanisms used by animals to cope with the varying structure of an environment in time (Kronfeld-Schor & Dayan, 2003; Sönnichsen et al., 2013). Environmental factors affecting temporal activity may include behavioural thermoregulation, avoidance of competitors, food resource availability and the avoidance of predators (Lesmeister et al., 2015; Pereira, 2010; Sönnichsen et al., 2013). If foxes perceive humans as predators, they should adjust their space use to human activities accordingly (e.g. Kaufmann et al., 2007). Our results show that foxes are mainly nocturnal and crepuscular. Beyond the stronger avoidance of highly populated areas during daytime, the selectin of sites inaccessible to humans is more distinct during times of high human activity. Such avoidance of human induced disturbance has been reported for a number of species that have shown an activity shift towards nocturnality across habitats and continents (Gaynor et al., 2018). Louvrier et al. (in prep) discovered that the city foxes of Berlin were more nocturnal during the recent covid lockdown and state that this is consistent with the expectation that people tended to be more present in their private gardens, forcing wildlife to be more nocturnal in the gardens, during lockdown. It seems that humans worldwide drive carnivores into the night (Carter et al., 2015; Sévêque et al., 2020), including red foxes, although prey activity is low during night-time (Díaz-Ruiz et al., 2016).

… and their limitations

Shifting activities to times of lower prey activity is a fitness disadvantage for foxes, as it limits temporal overlap with their prey and thus foraging opportunities. The cityscape provides food sources, reasonably independent of temporal patterns, yet the adjustment of foxes to human activity and space use comes with more evolutionary costs. Roughly 75% of foxes die in their first year, and thereafter mortality is approximately 50% in each adult year (Macdonald, 2004). Urban foxes are usually not hunted, they die from canine distemper virus, sarcoptic mange and other diseases, intraspecific competition as well as poisoning but trauma from traffic accidents is the most common cause of death.

Red fox mortality within cities is particularly high due to road traffic (Baker et al., 2007). Even if individuals survive traffic collisions, they are often severely injured. As a consequence, a high percentage of individuals in urban red fox populations have one or more fractures. For instance, one third of two to three year old red fox individuals and 70% of five year old individuals showed at least one fracture (Harris, 1978). In contrast, examinations of museum specimens of carnivores revealed a proportion of individuals with bone fractures of 4.4 % (Argyros & Roth, 2016). The high incidence of fractures is not surprising as we found that red foxes used transport infrastructures such as motorways for dispersal at the population level and even preferred grey spaces (mainly consisting of roads) over public green spaces and forests for individual habitat use.

The enormous risk that goes along with the use of these landscape elements indicates an erroneous risk estimation by the red foxes. Apparently, urban foxes show distinct predator avoidance behaviour towards humans, although urban wildlife species are not facing any direct mortality risk from humans, or more precisely from human pedestrians (Moll et al., 2018; Stillfried et al., 2017). There has been no hunting of red foxes in the Berlin city area for many fox generations, and poisoning of red foxes that is often caused by the consumption of (originally targeted) poisoned rodents is unlikely to be assigned to humans as the originator by the affected animals. We therefore propose an expansion of the concept of the landscape of fear (Laundré et al., 2010) on the ecological implications of fear induced behaviour beyond avoidance of natural predators to the consideration of human induced fears. The concept was originally developed for feeding behaviour (especially with regard to giving-up densities during foraging) and describes a landscape beyond physical properties, a landscape that “represents relative levels of predation risk as peaks and valleys that reflect the level of fear of predation a prey experiences in different parts of its area of use” (Laundré et al., 2010, S1). Such fears in wildlife may also arise from anticipated risks posed by humans, regardless of the actual assigned mortality risk. It has been shown that human induced fear or risk estimates by wildlife can indeed affect wildlife behaviour. For instance, top carnivores reduced fidelity to kill sites and overall consumption times in response to humans and compensated this by increasing foraging effort in terms of prey kill rates (Smith et al., 2015).

Our results suggest that the anticipation of risk related to direct human presence also drives red fox dispersal and space use behaviour, resulting in a costly misconception of risk levels in the landscape of fear. Laundré et al. (2010) postulate that animals can learn and respond to differing levels of risk. It remains an open question to what extent future red fox generations may improve the adjustment of their behaviour in response to humans.

Conclusion

Our research showed that the ecology of urban wildlife needs to be studied by considering human effects beyond manmade structures as an ecological factor. We demonstrated that red foxes are not entirely adjusted to the urban environment. Red foxes did not evenly use the urban matrix (without any preferences) on an individual scale nor was their population panmictic across the rural to urban gradient. Two distinct red fox populations were identified through clear genetic signatures and separated by the city border. Also, red foxes showed strong space use preferences for specific urban sites associated with reduced or missing human access and reduced human activities. Some physical properties of the landscape showed small barrier effects, but were not the main driver for or hindrance of red fox dispersal, in the sense of gene flow conductance and impediment. Hence, the red fox population is neither panmictic nor is dispersal solely affected by physical barriers. In terms of individual space use landscape composition also played a minor role, regarding the avoidance of landscape elements such as built-up areas as well as with regard to habitat preferences, in particular concerning the lack of preferential treatment of green spaces. Hence, behavioural constraints caused by human presence and activities may drive red fox dispersal, be responsible for structuring the population and set some limits to the use of space at the individual level.

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