



University of Natural Resources  
and Life Sciences, Vienna

# Spatial distribution and risk of potential hybridization of the European wildcat (*Felis silvestris silvestris*) with the Domestic cat (*Felis silvestris catus*) in Baden-Württemberg, Germany

## Master Thesis

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Vienna, October 2018





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### **Declaration in lieu of oath**

I herewith declare in lieu of oath that this thesis has been composed by myself without any inadmissible help and without the use of sources other than those given due reference in the text and listed in the list of references. I further declare that all persons and institutions that have directly or indirectly helped me with the preparation of the thesis have been acknowledged and that this thesis has not been submitted, wholly or substantially, as an examination document at any other institution.

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Date

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Signature

## **Acknowledgements**

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# 1 Abstract

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Natural hybridization is an important event in evolution and can lead to diversification and new evolutionary lineages. In contrast, introgressive hybridization induced by humans, in particular with our domesticated species, is a globally widespread phenomenon and can represent a threat to the native species' existence, fitness and genetic purity. In Europe, introgressive hybridization of protected carnivore species such as the European wildcat (*Felis silvestris silvestris*) with its domestic counterpart the Domestic cats (*Felis silvestris catus*) is an important topic of conservation concern. A recently recovering and expanding wildcat population is the one in the South-west of Germany where the species was considered to be extinct for over 100 years. This highly fragmented and relatively dense populated area of the Upper Rhine Valley in Baden-Württemberg is also home of many domestic cats. Free ranging domestic cats as well as wildcats share this area and potential hybridization of the two subspecies may represent a threat for the wildcats' genetic integrity. However, since the estimated hybridization rate of  $\sim 10\%$  is still low compared to other European wildcat populations some mechanisms seem to hamper unconfined reproduction of the two *Felis silvestris* subspecies. The main goal of this study was to assess if the presence of the European wildcat (*F.s. silvestris*) in forest habitats attracts or scares away the Domestic cat (*F.s. catus*) and thus gain insight into influencing factors and mechanisms of wildcat and domestic cat occurrences. In addition, the influence of anthropogenic factors on domestic cat occurrences in wildcat suitable habitat was investigated and their influence on a potential risk of hybridization. This study was conducted based on genetic data from 8 years of lure stick monitoring of the statewide wildcat monitoring in Baden-Württemberg conducted by the Forest Research Institute Baden-Württemberg and Bund für Umwelt und Naturschutz Deutschland. Data on domestic cat occurrences was obtained from overall 1642 lure sticks in subsequent years from 2008 to 2016. In addition 3749 data points from wildcat telemetry studies and data from 36 wildcat tissue samples were used to estimate wildcat occurrences. Within the framework of this thesis genetic laboratory analysis was conducted on samples collected in 2016. Wildcat and domestic cat samples were assigned to one of the two subspecies based on mtDNA and microsatellite fragment analysis. Landscape related variables were obtained from the ATKIS-Database, including forested areas, wildcat suitable habitat, settlement areas and data of single buildings outside of human settlement areas. For statistical analysis a binomial mixed model (GLMM) was chosen and calculated in R. Domestic cat records at the lure stick were selected as response variable, whereas data on wildcat occurrences, distance to settlements, size of settlements and distance to the nearest single building outside of settlement areas were chosen as predictors. The year of data collection was treated as random factor since every lure stick was located only once in the same area. Results of the statistical analysis displayed a significantly positive influence of wildcat occurrence in the close proximity to the lure stick on probability of domestic cat records ( $p = 0.00244$ , if wildcats were recorded within a distance of 100 m;  $p = 0.0372$ , if wildcats were recorded within a distance of 500 m). In addition the distance to settlements had a clear influence on domestic cat occurrences at the lure sticks. The probability of domestic cat records at a lure stick decreased

significantly with increasing distance to the settlement. Concerning the size of the nearest settlement, probability of a domestic cat record at the lure stick increased with growing size of the settlement until settlement reached an intermediate size. Beyond this value, occurrences of domestic cats at lure sticks declined again. In the surroundings of large settlements domestic cat occurrences at lure sticks were low again. Others than expected, the distance from lure stick location to the nearest single building outside of settlement areas did not show any significant influence on occurrences of domestic cats at lure sticks. The best explained variance ( $R^2_c = 0.285$ ) by fixed and random factors was achieved with the full model for the wildcat occurrence distance of 100 m and other predictor variables in its linear and quadratic terms. One of the main results of this thesis, that wildcat occurrences in the area seem to attract domestic cats within the close proximity to the lure stick, might be explained by the phenomenon of sex pheromone excretion during the mating season. In addition, small scale habitat structures within the close proximity of a lure stick might offer interesting requisites for wildcats as well as domestic cats and could additionally explain domestic cat occurrences at particular lure sticks. Still, the relatively low hybridization rate in the Upper Rhine Valley suggest, that some other underlying mechanisms might limit hybridization. One explanation could be the spatial organization of wildcat territories, whereat female wildcats seem to be concentrated inside the distribution range and might encounter less domestic cats. In the Upper Rhine Valley as well as in other agriculturally dominated landscapes female home ranges were notably smaller than home ranges of females in forested habitat. As confirmed by this and other studies, anthropogenic factors, such as the distance from lure stick location to the nearest settlement and the size of the nearest settlement, had significant influences on the encounter probability of species and suspected hybridization. To assess the potential risk of hybridization in detail, factors of influence from a small to a larger scale should be considered. To gain a deeper insight into the precise risk of hybridization between the European wildcat and the Domestic cat in Baden-Württemberg further analysis might be recommendable including detailed genetic analysis considering the sex of the individual as well as small scale habitat analysis at the particular lure spots. Moreover, the influence of roads and weather condition should be considered for interaction between wildcats and their domestic counterparts.

## List of abbreviations:

WK: European wildcat (*Felis silvestris silvestris*)

HK: Domestic cat (*Felis silvestris catus*)

BW: Baden-Württemberg

BUND: Bund für Umwelt und Naturschutz Deutschland

FVA: Forest Research Institute Baden-Württemberg

mtDNA: mitochondrial DNA (extranuclear double-stranded DNA found exclusively in mitochondria; is a circular molecule in most eukaryotes and maternally inherited)

PCR: Polymerase chain reaction (a laboratory technique used to make multiple copies of DNA segments)

bp: base pair (a unit consisting of two DNA nucleobases linked to each other by hydrogen bonds)

GLMM: generalized linear mixed model

## 2 Introduction

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The phenomenon of natural hybridization is an important event in evolution and can lead to diversification and new evolutionary lineages (Arnold & Hodges 1995). M.L. Arnold (1997) states in his book: "Natural hybridization involves successful matings in nature between individuals from two populations or groups of populations which are distinguishable on the basis of one or more heritable characters." Diverse studies already investigated natural hybridization occurring in all kind of organisms (Arnold 2004). While it is a widespread phenomenon in plants and birds (Grant & Grant 1992; Tovar-Sánchez & Oyama 2004; Zhang et al. 2017), in mammals it is less common, especially bearing the reproduction of fertile offspring (Larsen et al. 2010; Willis et al. 2004). However, in general it can lead to the formation of new lineages and speciation.

In contrast to natural hybridization, being part of the natural path of evolution, the phenomenon of hybridization induced by humans has its origin not very far back in the late Mesolithic and Neolithic Revolution (Driscoll et al. 2009). With the development of agriculture and large settlements humans started to cultivate grain and tame and domesticate different wild animal species. Worldwide different regions are known to be centers of domestication of our present-day livestock species such as sheep, cattle and goats (Diamond 2002; Zeder 2008). Regarding dogs and cats the path of domestication took a somewhat different development. Whereas livestock species were domesticated for food supply, dogs were initially proved useful as guards and hunting supporter, being the first species to be domesticated and adapted to humans. In contrast to this, cats were domesticated much later and their domestication is expected to be rather driven by natural selection (Driscoll et al. 2009). However, during the last millennia domesticated species were spread all around the globe going in line with agricultural expansion, human migrations and trade (Diamond 2002; Kalz 2001; Zeder 2008). Nowadays, hybridization of species or subspecies introduced by humans in a particular area is a globally widespread phenomenon and can represent a threat to the native species' existence, fitness and genetic purity (Biedrzycka et al. 2012; Muhlfeld et al. 2009; Tracey et al. 2008). Different studies gave indications that hybrid offspring for instance can have superior fitness compared to their progenitors and thus might be able to outcompete their parental phenotypes (Germain et al. 2008; Grant & Grant 1992; Zeder 2008) This problem could encompass in particular introgressive hybridization with our domesticated species (Giuffra et al. 2000; Randi 2008; Schröder et al. 2016).

In Europe, introgressive hybridization of protected carnivore species such as the Grey wolf (*Canis lupus*) and the European wildcat (*Felis silvestris silvestris*) with their domestic counterparts, domestic dogs (*Canis familiaris*) and domestic cats (*Felis silvestris catus*), is still a recent topic of conservation concern and subject of several studies (Hertwig et al. 2009; Oliveira et al. 2008; Pilot et al. 2018; Torres et al. 2017). Especially the European wildcat (*F. s. silvestris*), once widely spread throughout Europe seems to be susceptible to hybridization with the Domestic cat (*F. s. catus*) (Beaumont et al. 2001; Germain et al. 2008; Pierpaoli et al. 2003). Due to long term persecution, landscape fragmentation and habitat loss this elusive species is recently scattered in mostly isolated populations, thus hybridization with its domestic counterpart may threaten its genetic integrity (Mattucci et al.



2016; Yamaguchi et al. 2015). While extreme examples of highly introgressed wildcat populations occur in Scotland and Hungary (Beaumont et al. 2001; Pierpaoli et al. 2003), western European countries, such as France and Germany still denote a relatively low level of hybridization (Beugin et al. 2016; Eckert et al. 2009; Say et al. 2012).

Wildcat occurrences in Germany were recorded in two genetically distinct populations, the central German and the western German population, suggesting these populations to be isolated from each other (Birlenbach & Klar 2009; Eckert et al. 2009). More recent studies however detected a relatively continuous presence of the species with indications on genetic exchange between the two populations (Steyer et al. 2016; Tiesmeyer et al. 2018). The low level of hybridization ( $\sim 3\%$ ), estimated by these studies, indicate a relatively “healthy” wildcat population with low rates of introgression. In contrast to former studies suggesting expansion of wildcat ranges mainly by hybrids (Lecis et al. 2006), more recent studies revealed European wildcat range expansion without any direct human intervention and low levels of hybridization (Say et al. 2012). Hence, some natural mechanisms, such as direct outcompeting by aggressive territorial defense, are expected to take effect and restrict matings between wild and domestic cats also during the process of wildcats’ range expansion on population level.

A good example for a recently recovering and expanding wildcat population is the one in the South-west of Germany where the species could genetically be confirmed during the past 10 years (Streif et al. 2016). In the Upper Rhine Valley in Baden-Württemberg (BW), recolonization occurred presumably by dispersing individuals of the French and Swiss wildcat population (Nussberger et al. 2014; Würstlin et al. 2016). In this area the European wildcat was considered to be extinct for over 100 years. After two genetically confirmed wildcat carcasses were identified in 2006 and 2007 (Herdtfelder et al. 2007) a large scale monitoring program has been initiated to investigate wildcats’ distribution and occurrence. Since 2008 annual non-invasive lure stick monitoring is conducted by the Forest Research Institute Baden-Württemberg (FVA) with subsequent genetic hair analyses (Streif et al. 2016). Although the Upper Rhine Valley is a highly fragmented and relatively dense populated area, the estimated hybridization rate of  $\sim 10\%$  (Streif et al. 2016) is still low compared to other European wildcat populations. A mix of settlements, extensive agriculture and forestry, traditional architecture and scattered farms are shaping the landscape being home of many domestic cats (Ministerium für Wirtschaft 2018). Many free ranging domestic cats as well as wildcats share this area and potential hybridization of the two subspecies may represent a threat for the wildcats’ genetic integrity. However, considering the low hybridization rate in this region some mechanisms seem to hamper unconfined reproduction of the two *Felis silvestris* subspecies.

The main goal of this study was to assess if the presence of the European wildcat (*F.s. silvestris*) in forest habitats attracts or scares away the Domestic cat (*F.s. catus*) and thus gain insight into influencing factors and mechanisms of wildcat and domestic cat occurrences. On one hand, wildcats as many other species excrete special sex pheromones during the mating season (Gomez-Diaz & Benton 2013) that might also attract domestic cats in the vicinity. On the other hand, aggressive territorial behavior of wildcats within their home range boundaries might chase domestic cats off.

Another factor favoring hybridization might be the higher human population density in larger settlements and densely populated areas and thus more domestic cats. Nussberger et al. (2014) concluded in their study that areas with dense human population increase the opportunity of hybridization. Assuming that, hybridization risk might be expected to decrease with increasing distance to settlement. The consequences of these influencing factors might be an increased risk of hybridization or a low hybridization rate respectively.

In this context two hypotheses were phrased:

*H1: Proven occurrence of the European wildcat excludes occurrence of domestic cats in suitable wildcat habitats and potential risk of hybridization is low in this region.*

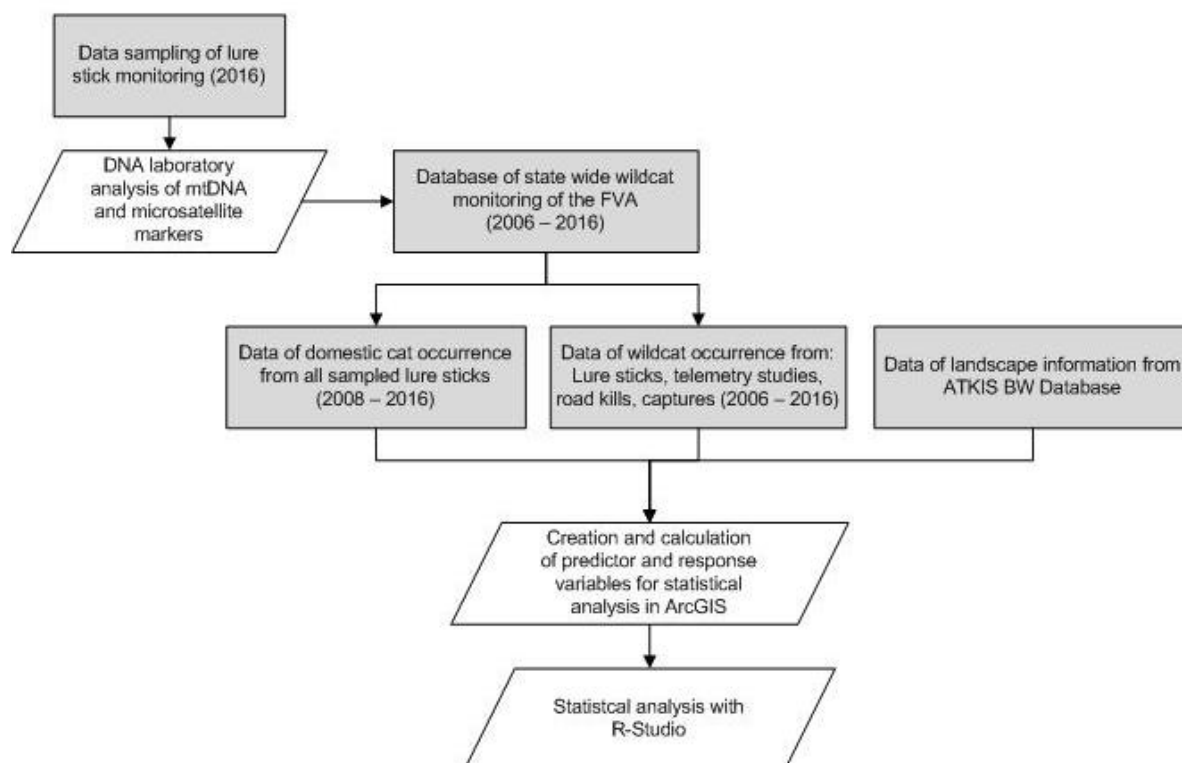
*H2: Occurrence of domestic cats depends mainly on distance to settlements or anthropogenic buildings outside of settlement areas and anthropogenic landuse.*

This study was conducted based on genetic data from 8 years of lure stick monitoring and landscape related variables.

### 3 Material & Methods

#### 3.1 Study design

This paragraph gives an overview of the study set-up. The flow chart in Figure 1 visualizes data sources (grey rectangles) and analyses steps (white rectangles). For this research the entire dataset of the statewide wildcat monitoring in Baden-Württemberg (BW) conducted by the Forest Research Institute Baden-Württemberg (FVA) and Bund für Umwelt und Naturschutz Deutschland (BUND) was used. Data sampling was conducted in cooperation with other local institutions (see Appendix 1 for detailed information on participating institutions).



**Figure 1: Flow chart of the study design with an overview of the set-up and working steps. The figure displays data sources (grey rectangles) as well as different process steps (white rectangles).**

Within the framework of this thesis laboratory analyses on mitochondrial DNA (mtDNA) and microsatellite markers were carried out for hair and tissue samples collected in 2016 (see chapter 3.5). Genetically proven occurrences of wildcats or domestic cats were then added to the FVA- intern genetic database. Variables for further statistical analysis were created with data of domestic cat occurrences at lure sticks (variable HK; see Table 1) and wildcat occurrences (from lure sticks, telemetry studies, road kills and captures). Wildcat records within a particular distance to the lure stick were subdivided into 6 distance categories resulting in six binomial variables (variables WKin100 - WKin2000). In addition landscape information based on ATKIS (ATKIS - Amtliches Topographisch-Kartographisches Informationssystem Version 6.0 BW, 2011) was used to calculate variables for the distance from lure stick location to the nearest settlement, the size of the nearest settlement and the distance from lure stick location to the nearest single building outside of settlement areas (variables DistLStSiedl, Areaqkm and DistLStEinzelBau respectively). The year of lure stick data collection

(variable Jahr) was included in the analysis, since data collection occurred in annually shifting areas. Variables for further statistical analysis were created in ArcGIS version 10.4.1 (Esri ArcGIS, 2017).

**Table 1: Variables used in this study for the final statistical analysis. The variables 1 and 3 (3a – 3f) are based to domestic cat and wildcat occurrences respectively; the variables 4 – 6 are based on landscape information; variable 2 is the year of lure stick data collection.**

Variable Number	Variable code	Variable description
1	HK	Domestic cat record at the lure stick
2	Jahr	Year of lure stick data collection
3	WKin100 - WKin2000 (6 different variables)	Wildcat record within a particular distance to the lure stick
3a	WKin100	Wildcat record within a distance of 100m to the lure stick
3b	WKin500	Wildcat record within a distance of 500m to the lure stick
3c	WKin1000	Wildcat record within a distance of 1000m to the lure stick
3d	WKin1200	Wildcat record within a distance of 1200m to the lure stick
3e	WKin1500	Wildcat record within a distance of 1500m to the lure stick
3f	WKin2000	Wildcat record within a distance of 2000m to the lure stick
4	DistLStSiedl	Distance from lure stick location to the nearest settlement
5	Areaqkm	Size of the nearest settlement
6	DistLStEinzelBau	Distance from lure stick location to the nearest single building (outside of settlement areas)

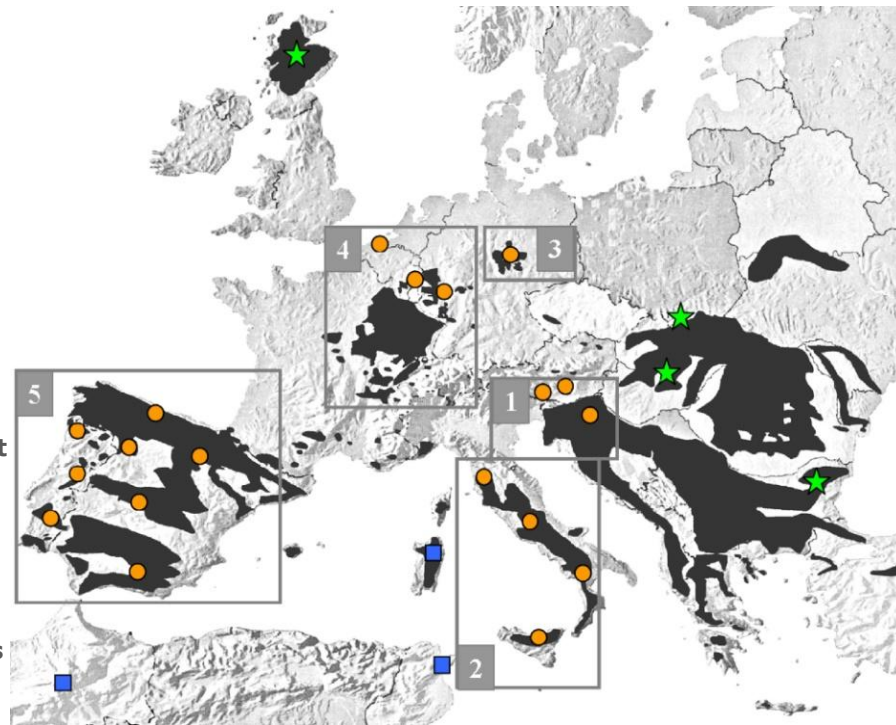
Detailed information on the particular processing steps, encompassing data sampling, laboratory analysis, ArcGIS and statistical analysis are specified in the chapters 3.4 - 3.8.

## 3.2 Study species´

### 3.2.1 The European wildcat (*F. s. silvestris*)

The European wildcat (*Felis silvestris silvestris* Schreber, 1777) is one of five *Felis silvestris* subspecies distributed worldwide (Driscoll et al. 2007; Yamaguchi et al. 2015). In its historic range the European wildcat was distributed in most parts of the European continent but due to severe human induced population declines especially in the early 19<sup>th</sup> century European wildcats are recently scattered in more or less fragmented populations (Figure 2) (Nowell & Jackson 1996; Piechocki 2001).

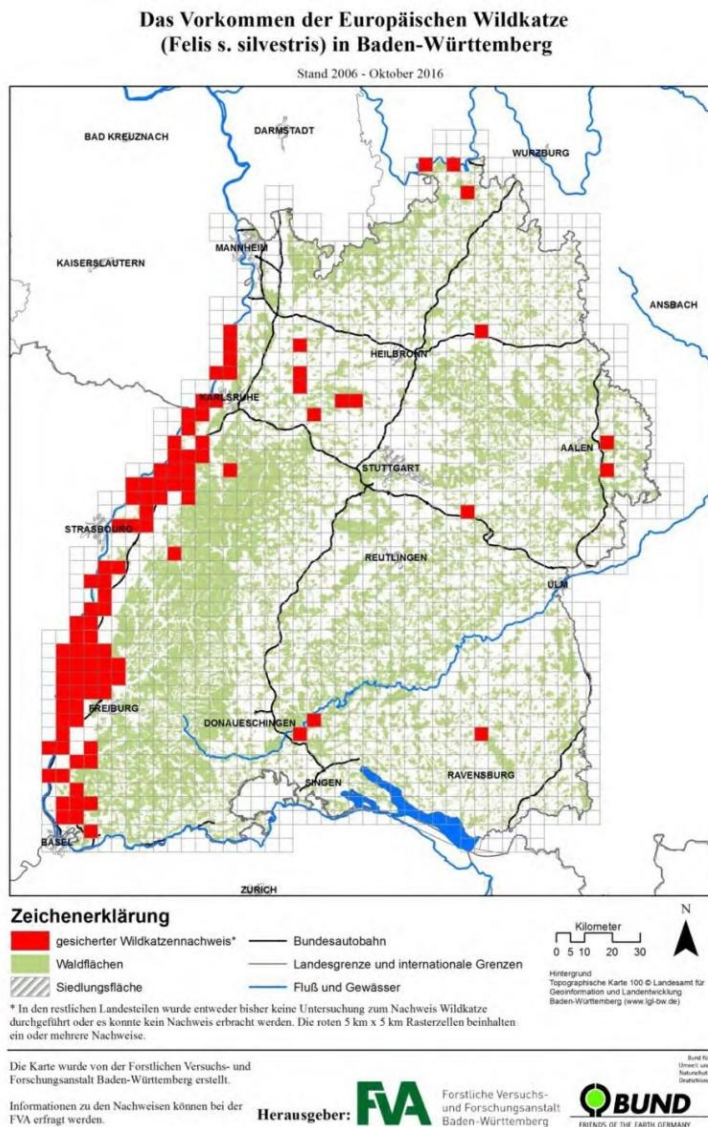
**Figure 2: Distribution of five European wildcat (*F. s. silvestris*) biogeographic groups identified through multivariate and Bayesian cluster analyses (numbered squares 1-5); dark areas represent the approximate distribution of wildcats (*F. s. silvestris*); orange dots: approximate sampling locations; green stars: rough locations of the admixed European wildcat populations in eastern Europe and introgressed domestic (*F. s. catus*) x European wildcat populations in Hungary and Scotland; blue squares: sampling regions of African wildcats (*F. s. libyca*). Source of map: Mattucci et al. (2016)**



In Germany two genetically distinct wildcat populations are distributed in Rhineland-Palatine and North Rhine-Westphalia (western German population) and in Lower Saxony, Saxony-Anhalt and the North of Thuringia (eastern German population), as visualized by cluster 3 & 4 in Figure 2 (Hertwig et al. 2009; Steyer et al. 2016). In the southern part of Germany, especially in BW the European wildcat was considered to be extinct since 1912 and was therefore listed in Category 0 on the national Species Red List (Braun & Dieterlen 2003). Since 2006 / 2007 the species was considered to be redetected due to two wildcat carcasses from road accidents (Herdtfelder et al. 2007).

The recent distribution of the European wildcat in BW is documented mainly along the Upper Rhine Valley from Lörrach at the Swiss border to the north of Karlsruhe (Figure 3). Adjacent populations of wildcats around this area are distributed in the western Vosges Mountains (France), the Alsace Rhine plain (France), the Palatine Forest (Germany) and the Jura area (Switzerland). Single European wildcat records in the North and East of BW indicate migrating wildcats from Bavaria, Hesse or Rhineland-Palatine (Streif et al. 2016).

Based on its ecology and habitat requirements the European wildcat has become an umbrella and flagship species to promote large, connected broad-leaved forests and reconnection of forest patches by wildlife corridors (Trinzen & Klar 2010; Vogel et al. 2009). Although the species often was classified as strictly forest dependent (Götz 2015; Guggisberg, C. A. W, Ragni, B., cited in Lozano 2010), recent studies also indicate European wildcat occurrence in extensive used landscapes, shrubland mosaic, meadows and near watercourses, as long as coverage and prey availability match the species requirements (Götz 2015; Klar et al. 2008; Lozano 2010).



**Figure 3: Distribution map of the European wildcat in BW in 2016. Red squares display confirmed wildcat occurrences within the 5x5 km raster. The data is based on a national wide monitoring program of FVA and BUND. Source of map: Streif S. et al. (2016)**

Western Germany home range sizes up to 3566 ha were calculated (Dietz et al. 2016). Most of these studies indicate differences between home range sizes of females and males, whereat males having significantly larger home range sizes than females and the home range of one male can overlap those of several females. In addition differences in home range sizes between mainly forested areas and open landscapes with forest patches, meadows, and agricultural extensive used landscapes could be distinguished. In the Upper Rhine Valley Götz et al. (in Press) defined two categories of wildcats using more "open-land-habitat" or mainly "forested habitat". Estimations of home ranges in this area resulted in minimum required habitat availability for annual home ranges of:

- a) "open-land-habitat"- wildcats: 1.100-1.200 ha (males) & 200-300 ha (females)
- b) "forested habitat"- wildcats: 1.200 ha (males) & 500 ha (females)

Home ranges in this area also encompass anthropogenic used landscapes and may comprise areas of interaction between the European wildcat and the Domestic cat.

These results are also confirmed by telemetry studies in the densely populated and fragmented area of the Upper Rhine Valley in BW. In the Southern part of the Upper Rhine Valley Streif et al. (2016) pointed out that small scale habitat utilization of the European wildcat also comprises fallow land, traditional orchards or industrial areas of gravel plants. Similar to other studies in Germany difference could be detected in small scale habitat utilization between sexes. While female wildcats use predominantly forested habitat in their activity ranges, males more often include open land, agricultural field margins and drainage channels (Dietz et al. 2016; Thiel 2004).

Home ranges sizes of the European wildcat depend on habitat types, its availability and prey densities (Dietz et al. 2016; Götz & Roth 2007; Lozano 2010). Estimated sizes can vary between 122 ha (Germain et al. 2008) and 2902 ha (Götz & Roth 2007). In the Rothaargebirge in

### 3.2.2 The Domestic cat (*F. s. catus*)

In contrast to historic assumptions of first cat domestication in ancient Egypt, more recent studies state first signs of domestication already ~ 9500 years ago in Cyprus and ~ 5500 years ago in China (Hu et al. 2014; Vigne et al. 2004). Genetic studies on the origin of the Domestic cat (*Felis silvestris catus*) revealed the Near Eastern wildcat (*Felis silvestris lybica*) to be the maternal ancestor (Driscoll et al. 2007). Initial traces of domestication have different geographic centres of origin - the Near East and Egypt - as well as different historical times (Ottoni et al. 2017). Driscoll et al. (2009) estimate a time frame of cat domestication between 9300 – 3600 B.P. with first separations of *Felis silvestris lybica/ catus* mtDNA clade already 131,000 years ago. In contrast to domestication of wolves/ dogs, first traits of cat domestication are based on natural selection coinciding with the development of year-round settlements and agriculture in the Neolithic. Later on cats were traded as valuable presents, or used as mouse hunters on ships, leading to a worldwide distribution (Kalz 2001). Nowadays the Domestic cat, being the most popular pet worldwide, occurs almost everywhere and in varying contact to humans; from pure “indoor cats” without any access to roam freely to “feral cats” without almost any contact to humans (Liberg et al. 2000). For this study however only free ranging domestic cats with a particular owner were considered, since study sites are located in a relatively dense populated area and include the vicinity to human settlements and farms.

During the last decades the Domestic cat became an interesting subject to wildlife- and ecological studies. Behavior, habitat utilization, home range sizes and human-related factors of domestic cats were investigated; particularly with regard to competition or hybridization with the threatened European wildcat (Biro et al. 2004; Ferreira et al. 2011; Germain & Poulle 2012; Liberg et al. 2000). Being a human attached species, home ranges of the Domestic cat are often centered at farms or other anthropogenic buildings, as figured out by studies in Spain and France (Ferreira et al. 2011; Germain et al. 2008). Space utilization in general is mainly linked to distances to their homes, food sources or shelter (Liberg et al. 2000; Spotte 2014). In contrast to the European wildcat, that has special requirements to its habitat, the Domestic cat roams throughout all types of landscapes. Nevertheless Metsers et al. (2010) found preferences in habitat utilization in two of three study areas, showing that domestic cats avoid habitat with little or no cover, such as cultivated farmland or duneland.

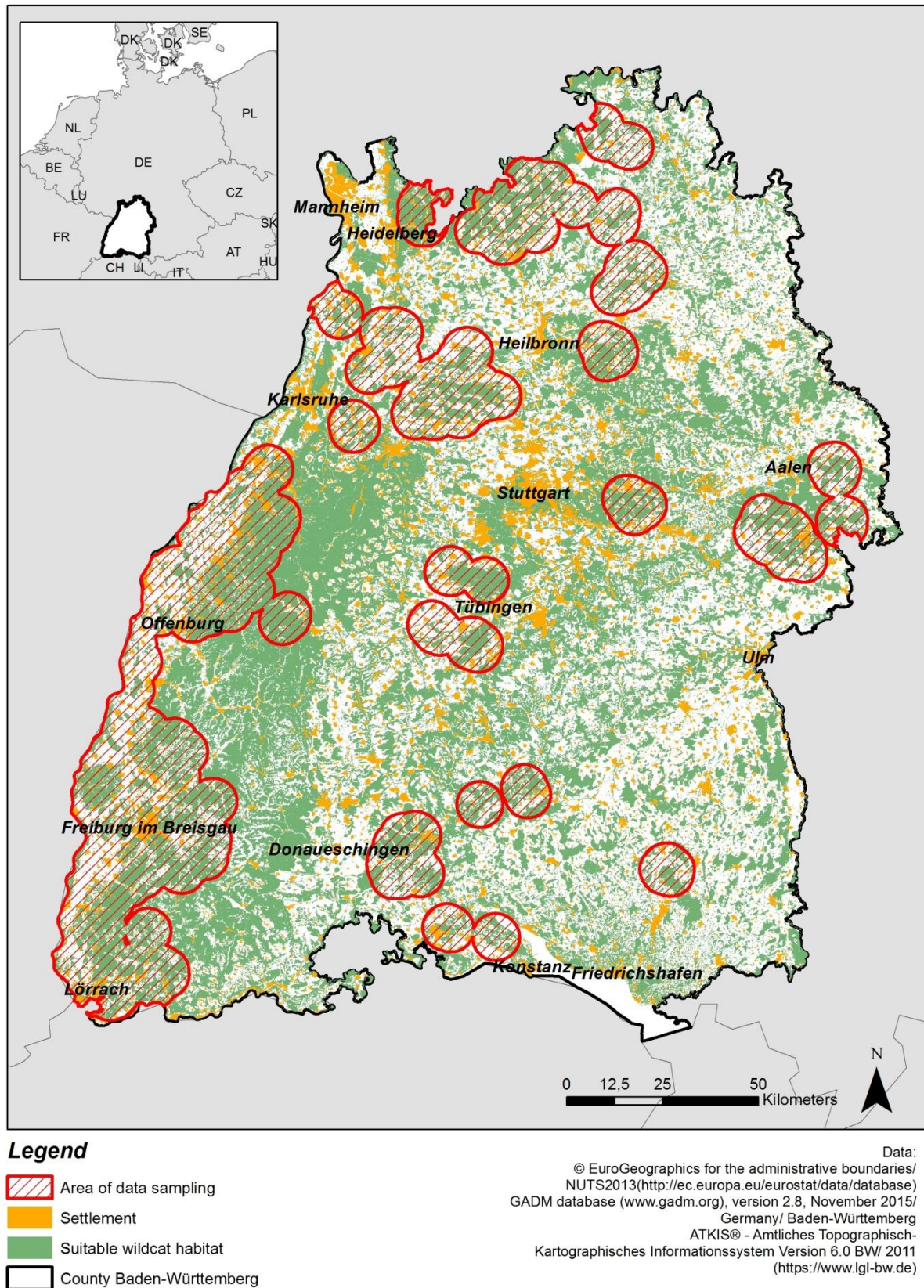
Although home ranges of domestic cats show large variations in size (between 2 and 358 ha) they are mostly smaller than those of the European wildcat (Germain et al. 2008). Metsers et al. (2010) estimated home ranges of domestic cats at rural sites being larger than those of cats living at urban edges and show differences in size between day and night. Especially fertile males of the Domestic cat have significantly larger home ranges, mainly to cover several female home ranges (Kitts-Morgan et al. 2015). This spatial ranging pattern however, can change between mating and non-mating season (Liberg et al. 2000). In areas where wild and domestic cats occur, overlapping home ranges do not seem to be a necessary prerequisite for hybridization of the two subspecies (Biro et al. 2004; Germain et al. 2008).

### 3.3 Study area

The study encompasses the entire period of data collection within the scope of the statewide European wildcat monitoring conducted by the FVA and BUND. The study area comprises the county of BW and individual sampling sites were initially selected based on suspected wildcat occurrences in the particular areas. However, at several sampling sites wildcat occurrences could not be genetically confirmed (Figure 4).

The largest connected study site was located along the Upper Rhine Valley from the Swiss- German border in the south up to the city of Karlsruhe in the north. With an altitude of 85 – 245 m NHN the area lies between the Vosges Mountains in France and the Black Forest Mountain Range in BW. It encompasses the "Markgräflerland" region to the south and the "Kaiserstuhl" region to the west of Freiburg im Breisgau (Röhr 2017). In the northern part of BW study sites were located in the "Neckar- und Tauberland, Kraichgau, Hohenlohe" region to the north and west of Heilbronn and in the adjacent "Odenwald, Spessart & Südrhön" at the border to Hesse. In the central and southern part of BW study sites near Tübingen and Donaueschingen were located in the "Schwäbisch-fränkisches Keuper-Lias-Land", "Schwäbische Alb" and "Alpenvorland" regions, respectively. (LUBW 2017)





**Figure 4: Map of the entire study area of this research encompassing the county of Baden-Württemberg. Red shaded zones indicate the areas of lure stick data sampling.**

### 3.4 Data sampling

The entire dataset used for this study comprised 899 hair samples from wildcats ( $n = 593$ ) and domestic cats ( $n = 306$ ). It was obtained from the database of statewide European wildcat monitoring conducted FVA and BUND. Samples were collected from overall 1642 lure sticks in subsequent years from 2008 to 2016 (Table 2). Lure sticks are rough wooden sticks with a dimension of 60 (100) x 4.8 x 2.4 cm first used for wildcat hair sample collection by Hupe in 2004 (Hupe & Simon 2007). To attract wildcats and improve sampling success the sticks were soaked with pure valerian tincture. All hair samples were collected in annually shifting areas according to the non-invasive lure stick method described in Steyer et al. (2012). The lure sticks were controlled in a weekly interval by skilled persons according to a specified protocol (Weber et al. 2008).

**Table 2: Number of placed lure sticks per year for data collection of European wildcat occurrences. The lure sticks were placed in annually shifting areas.**

Year of data collection	2008	2009	2010	2011	2012	2013	2014	2015	2016
Number of placed lure sticks	131	48	215	152	230	193	181	178	314

Additionally 3749 data points from wildcat telemetry studies conducted by FVA during a period of four years (2009 - 2012) and data from 36 wildcat tissue samples, obtained from carcasses or catches (FVA, 2006 - 2016), were used for calculation of the variable "Wildcat record within a particular distance to the lure stick" (WKin100 - WKin2000; see Table 1).

Samples collected in 2016 were processed in the framework of this thesis comprising 414 hair samples ( $n = 406$  FVA-MOBIL Projekt;  $n = 8$  Naturpark SFW) and 8 tissue samples. Hair samples were collected from 300 lure sticks (January - March 2016) in the Markgräflerland region (FVA-MOBIL Projekt) and 14 lure sticks in the Naturpark SFW region. Tissue samples were obtained from cat carcasses due to road kill events.

### 3.5 Laboratory analysis

Laboratory analysis was conducted in the FVA in-house laboratory for all collected samples in 2016 according to a standardized procedure. Extracted DNA was used for mtDNA and microsatellite analysis to identify wildcat and domestic cat individuals. Prior to laboratory work all hair samples ( $n = 414$ ) were stored in envelopes in the dark. Tissue samples were stored in 96% ethanol, frozen at  $-20^{\circ}\text{C}$ . The next paragraphs (3.5.1 - 3.5.4) give a detailed description of the processing steps including isolation of DNA, preparation for mtDNA analysis and microsatellite fragment analysis.

#### 3.5.1 DNA isolation

For DNA extraction only hair samples with a minimum of 3 - 5 hairs with clearly recognizable roots and about 10 undercoat hairs were used ( $n = 265$ ). Hair roots were cut off under the microscope at a maximum length of 1 cm, while undercoat hairs were used entirely for extraction. DNA of hair roots and tissues was extracted using the QIAamp DNA MicroKit (Qiagen, Hilden, Germany) according to

the manufacturer's protocols. The extracted DNA both from hair roots and tissue samples was eluted in 60 µl AE buffer and stored at -20 °C until polymerase chain reaction (PCR) and further analysis.

### 3.5.2 mtDNA preparation

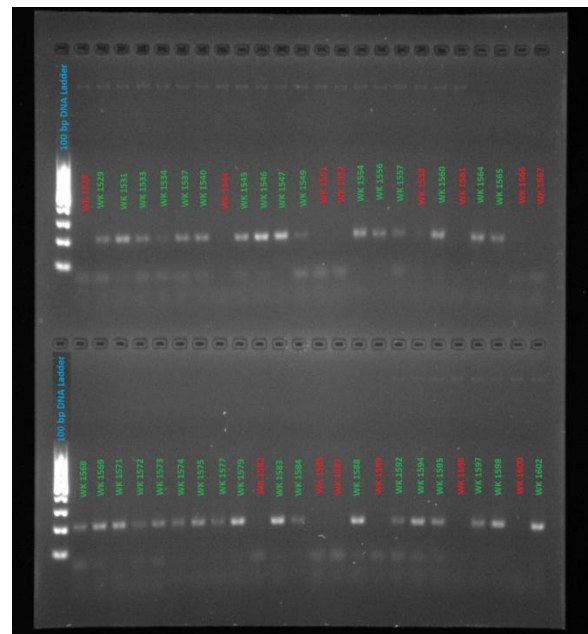
For mtDNA analysis in this study the M3 control region with around 200 base pairs (bp) was amplified according to a validated protocol. PCR was conducted for amplification, using the primers L4f (forward: 5'-GACATAATAGTGCTTAATCGTGC-3') (Eckert et al. 2009) and DLH (reverse: 5'-CCTGAAGTAAGAACCAGATG-3') (Tiedemann et al. 1996). PCR volume of 20 µl/ sample contained: 0.3 µM of each primer, 0.2 mM dNTP, 1.5 mM MgCl<sub>2</sub>, 20 mM Tris-HCl (pH 8.3), 100 mM KCl, 0.03 Units of SuperHot Taq Polymerase, 11.06 µl H<sub>2</sub>O and 4.0 µl of DNA.

PCR was run at a program for mtDNA amplification with the following steps: preheat and initial denaturation at 95 °C for 15 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s. Subsequent a final extension at 60 °C for 30 min was carried out at the end of the program and a constant cooling at 10 °C.

### 3.5.3 Gel electrophoresis

For initial identification of felid DNA a gel electrophoresis was run on agarose gel. Therefore 3 µl PCR product of amplified mtDNA fragments were added to 5 µl 6X DNA Loading Dye (Thermo Scientific, Waltham, MA USA) for easy visual tracking of DNA migration during electrophoresis. Gel was prepared of 100 ml TAE buffer with 1.5 g Agarose LE powder adding 8 µl GelRed (TM) (Genaxxon bioscience, Ulm, Germany) fluorescent dye to stain DNA fragments in the agarose gel. Each gel contained 50 wells for 48 DNA samples and 2 DNA ladders. The GeneRuler 100 bp DNA Ladder (Thermo Scientific, Waltham, MA USA) was used as reference for sizing and approximate quantification of DNA fragment length. Gel electrophoresis was run at 100 - 120 mA for 30 minutes. Subsequently the result was visualized using a UV light-box and the Software GeneSys (Syngene, Cambridge, UK).

For further mtDNA analysis only samples of felid DNA were selected (Figure 5). The mtDNA was filtered and prepared for sequencing by addition of 1.5 µl ExoSAP-IT (GE Healthcare Life Sciences, München) to 5 µl PCR-mix and short term denaturation (annealing at 37 °C for 15 min and denaturation at 80 °C for 15 min). Sequencing was carried out by LGC Genomics GmbH, Berlin.



**Figure 5: Gel electrophoresis result of 48 DNA samples (WK1528 – WK1602) visualized with the Software GeneSys (Syngene, Cambridge, UK). Luminescent DNA fragments (green labelled) indicate felid DNA non-luminescent samples (red labelled) are non-felid DNA.**

### 3.5.4 *Microsatellite fragment analysis*

Microsatellite genotyping was based on a validated set of 14 microsatellite markers (FCA 571, FCA 8, FCA 124, FCA 171, FAC 149, FAC 275, FAC 88, FAC 170, FAC 364, FAC 576, FAC 132, FAC 567, FAC 232, FAC 347) and one sex-marker (ZF) (Menotti-Raymond et al. 1999; Pilgrim et al. 2005). Partially labeled (forward) and unlabeled (reverse) primers were mixed according to a given protocol resulting in a primer mix with a total concentration of 20  $\mu$ M for all primers. Microsatellite markers were combined on 4 multiplex PCRs. To ensure analysis success and avoid sequencing dropout three replicates per sample were carried out. 10  $\mu$ l PCR mix per sample contained: 3.8  $\mu$ l extracted DNA, 5  $\mu$ l 2x HotStart Mastermix (Genaxxon bioscience, Ulm, Germany) and 0.3 – 0.4  $\mu$ l of each primer mix. PCR was run for all multiplex with the following steps: denaturation at 95 °C for 15 min, 31 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 1 min, extension at 72 °C for 1 min with a final extension at 72 °C for 30 min. At the end of the program and a constant cooling at 4 °C was carried out. Prior to fragment analysis PCR products were prepared with 13.9  $\mu$ l HiDi (Formamid) and 0.2  $\mu$ l LIZ(500) size standard per 1  $\mu$ l PCR product and subsequently denatured for 5 min at 95°C. Fragment analysis was run on an ABI 3130 DNA Analyzer (Applied Biosystems, Darmstadt, Germany).

## 3.6 Genetic data analysis

Data correction and alignment of the obtained mtDNA sequences was done using the program MEGA version 6.06 (MEGA – Molecular Evolutionary Genetics Analysis, 2016). Subsequently a phylogenetic tree with the Neighbour-Joining (NJ) distance method was created resulting in distinct domestic and wildcat haplotype clusters. A dataset of 45 different wildcat and domestic cat haplotypes from former studies was used as reference. The cat individuals used as reference were previously determined genetically and morphologically by FVA and Senckenberg Research Institute, Frankfurt, Germany (Würstlin 2013).

Fragment lengths of microsatellites were coded relative to the size standard using GeneMapper 4.0. (Applied Biosystems, Darmstadt, Germany) and consensus was done manually for all three replicates of each sample. Genotypes were accepted, if at least two out of three replicates showed the same results in alleles. Microsatellite analysis was carried out using the program STRUCTURE version 2.3.4 (Pritchard et al. 2000). Samples were run together with a reference set of domestic cat (n = 74) and wildcat (n = 19) genotypes, also determined genetically and morphologically by FVA and Senckenberg Research Institute, Frankfurt, Germany (Würstlin 2013). A genetically proven affiliation to the wildcat population cluster was considered if the probability of Q (individual proportion of membership) was > 0.8. Other samples with a proportion of > 0.2 were assigned to the domestic cat cluster (Pierpaoli et al. 2003; Oliveira et al. 2008). The results were then added to the FVA-intern genetic database prior to further processing.

### 3.7 GIS data selection

Selection and calculation of statistical variables was done in ArcGIS version 10.4.1 (Esri ArcGIS, 2017) and landscape data was obtained from the ATKIS-Database (ATKIS - Amtliches Topographisch-Kartographisches Informationssystem Version 6.0 BW, 2011).

Initially the following input data was used to create and calculate variables:

- 1) Table of all lure stick locations (n = 1642) during the monitoring period 2008 - 2016 with information on domestic cat records
- 2) Table of all wildcat records collected during the period of 2006 - 2016 from: telemetry studies (n = 3749), lure sticks (n = 593), single captures (n = 2), road kills (n = 34)
- 3) ATKIS landscape data:
  - a. data of settlement areas
  - b. data of single buildings outside of human settlement areas
  - c. data of forest areas in BW
  - d. data of suitable wildcat habitat additionally to forest areas

Data of forest and settlement areas in BW were directly extracted from the basic ATKIS-Database. Single buildings outside of settlement areas and suitable wildcat habitat in addition to forested areas were selected according to Ehrhart (2015) and altered with categorical adaptations for the whole county of BW (see Appendix 2). Subsequently, data of forest areas and secondary suitable wildcat habitat were merged together to one layer. A buffer of 100 m around the layer was created to expand suitable wildcat habitat since wildcats are also known to use forest edges for e.g. hunting (Götz et al. in Press; Herrmann & Klar 2007; Klar et al. 2008). To facilitate the calculation process in ArcGIS the entire landscape data was reduced to an area of 10 km around lure stick and wildcat occurrence positions.

#### 3.7.1 Domestic cat data

The entire table of lure stick locations (n = 1642), set up during the monitoring period 2008 – 2016, was selected as base for creation and calculation of all other variables. Linked to this table domestic cat records at the lure stick were created as binomial variable and coded as "0 = no domestic cat (HK) record at the lure stick" or "1 = record of domestic cat at the lure stick".

#### 3.7.2 Wildcat occurrence variables

Initially the dataset of wildcat records collected during the period of 2006 - 2016 was split up into A) "telemetry data" and B) "all other types of wildcat records" to facilitate calculation process. Occurrence of wildcats in a particular distance to every single lure stick were created as binomial variables and coded as "0 = no WK record within the particular radius to the lure stick" or "1 = WK record within the particular radius to the lure stick". To code the variables related to lure stick locations buffers with 6 distinct distances around every wildcat record were created (100 m, 500 m, 1000 m, 1200 m, 1500 m, 2000 m) following indications on wildcat home range sizes and activity ranges (Biro et al. 2004; Klar 2009; Götz et al. in Press). Thereafter buffers were clipped to suitable wildcat habitat to avoid selection errors and exclude unsuitable lure stick locations from further analysis. Wildcat record variables were created separately for every single distance respectively

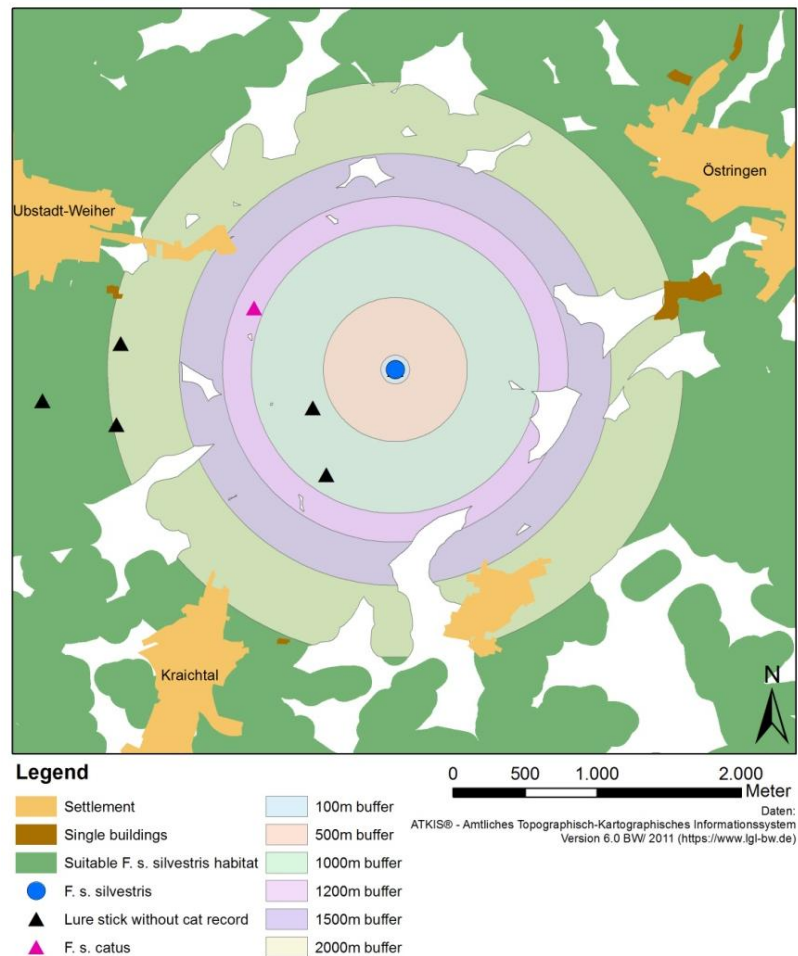


resulting in 6 variables for dataset A) and 6 variables for dataset B). Figure 6 displays an example of the created buffers of wildcat occurrence and lure stick locations for variable estimation.

To avoid calculation errors of wildcat occurrence in the proximity of a lure stick data was selected by year; only wildcat records in the same year or previous years to lure stick data sampling were coded with 1 in the particular buffer distance. The 12 binomial variables (6 variables from A) telemetry data and 6 variables from B) all other types of wildcat records) were linked to the attribute table of the lure stick locations.

Before implementation of the

dataset in R wildcat occurrences in a specific radius (100 m; 500 m; 1000 m; 1200 m; 1500 m and 2000 m) to the particular lure stick were merged together from the two categories A) and B).



**Figure 6: Buffer selection of wildcat occurrence in a particular distance to a lure stick. The blue dot represents occurrence of a wildcat, pink and black triangles show lure stick locations with (pink) or without (black) domestic cat records.**

### 3.7.3 Landscape related variables

Based on data of settlements and single buildings outside of human settlement areas in spatial relationship to the lure sticks the variables "Distance from lure stick location to the nearest settlement" and "Distance from lure stick location to the nearest single building" were calculated. Additionally the size of the nearest settlement within the selected study area was estimated in km<sup>2</sup>. These three variables were also linked to the attribute table of the lure stick locations. Table 3 displays the final selection of relevant variables for the statistical analysis with a detailed description of each variable. A detailed flow chart of the discrete processing steps in Arc GIS is attached in Appendix 3.

**Table 3: Input dataset of relevant variables for statistical analysis in R. Variables were created and calculated in ArcGIS version 10.4.1.**

Variable code	Format	Description	Comment
<b>HK</b>	Factor	Domestic cat record at the lure stick	„0 = no HK“ „1 = HK“ Every result others than HK at the lure stick was coded with “0”
<b>Jahr</b>	Scale	Year of lure stick data collection	
<b>LSt_ID</b>	Scale	Individual ID for dataset created from x- und y-coordinate	“x-coordinate” * “y-coordinate” = individual ID
<b>WKin100</b> - <b>WKin2000</b> (6 different variables)	Factor	Wildcat record within a particular distance to the lure stick	„0 = no WK record within the particular radius“ „1 = WK record within the particular radius “ LSt-data (lure stick locations) was selected by year; only WK records of the same year or previous years than lure stick sampling were coded with 1 if within the buffer radius.
<b>DistLStSiedl</b>	Scale	Distance from lure stick location to the nearest settlement	In meter
<b>Areaqkm</b>	Scale	Size of the nearest settlement	In km <sup>2</sup>
<b>DistLStEinzelBau</b>	Scale	Distance from lure stick location to the nearest single building (outside of settlement areas)	In meter Categories comprise anthropogenic used buildings and areas like: single farms/ farm buildings, camping areas, holiday cabins, garden plots, cemetery etc.

### 3.8 Statistical analysis

Statistical analysis was carried out with RStudio version 1.0.136 (RStudio Inc © 2009 - 2016) based on R version 3.3.3 (R-3.3.3 for Windows, 2017).

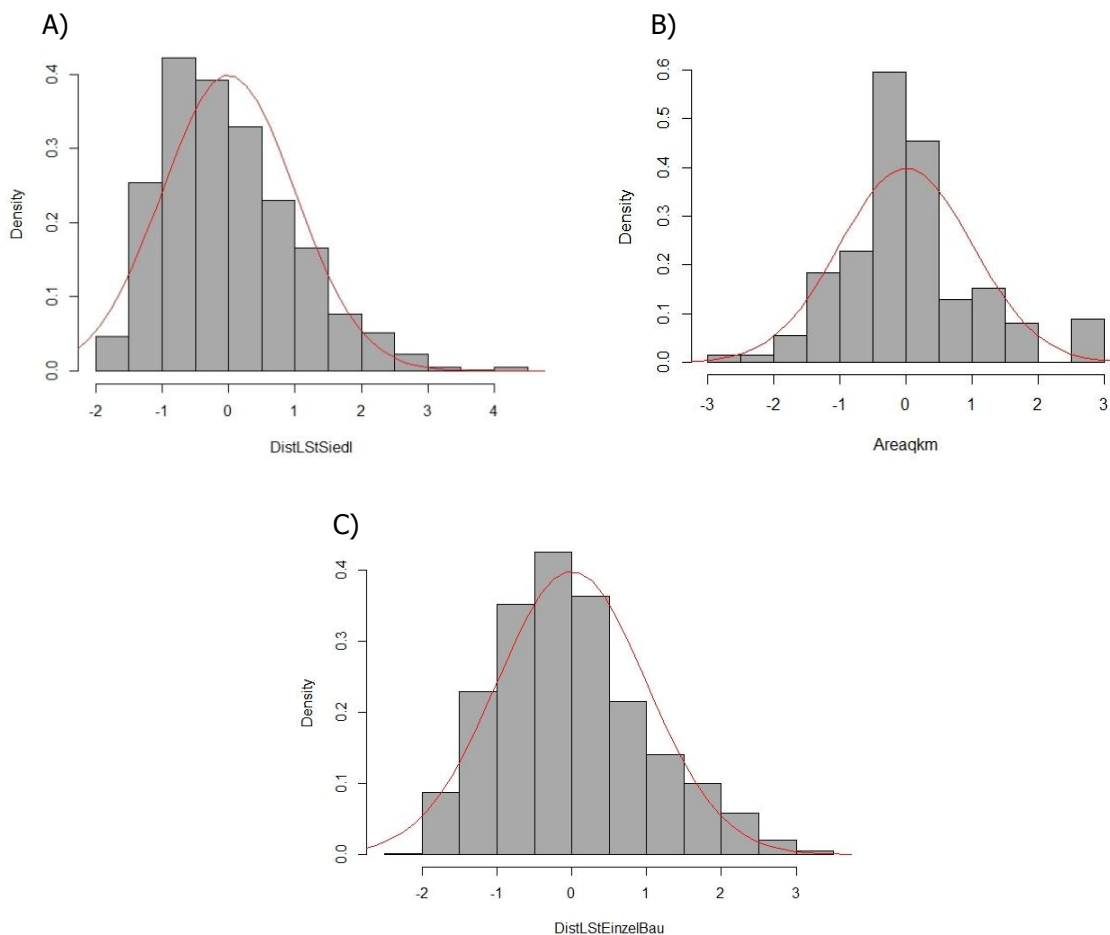
To test the hypotheses of this study a binomial mixed model (GLMM) was chosen (Bolker et al. 2009). The most important input variables comprised: “Domestic cat record at the lure stick” (HK), “Year of lure stick data collection” (Jahr), “Wildcat record within a particular distance to the lure stick” (WKin\*\*), “Distance from lure stick location to the nearest settlement” (DistLStSiedl), “Size of the nearest settlement” (Areaqkm) and “Distance from lure stick location to the nearest single building” (DistLStEinzelBau) (Table 3). As response variable for the GLMM “Domestic cat record at the lure stick” was selected, the other variables were used as predictors (DistLStSiedl, Areaqkm, DistLStEinzelBau). The variable “Year of lure stick data collection” (Jahr) was treated as random factor in the binomial mixed model, since every lure stick was located only once in the same area, which means only one sampling period per year in one particular area.

The initial models were:

$$\text{HK} \sim \text{WKin}^{**} + \text{DistLStSiedl} + \text{Areaqkm} + \text{DistLStEinzelBau} + (1 \mid \text{Jahr})$$

\*\* For every wildcat record distance to the lure stick a separate model was created, resulting in 6 different initial models for the distances 100 m, 500 m, 1000 m, 1200 m, 1500 m and 2000 m respectively.

Prior to model building the variables “Domestic cat record at the lure stick” and “Wildcat record within a particular distance to the lure stick” were transformed into factor. Collinearity of variables was tested by pairwise Pearson correlation for all scale variables (Jahr, DistLStSiedl, Areaqkm, DistLStEinzelBau). Distribution of the data for each numeric predictor variable was visualized using histograms. For the variable “Size of the nearest settlement” a log-transformation turned out to be reasonable and all predictor variables were standardized with z-Transformation (Figure 7).



**Figure 7: Histograms of adjusted predictor variables prior to statistical modelling;**  
**A) standardized “Distance from lure stick location to the nearest settlement” (LStDistSiedl),**  
**B) log transformed and standardized “Size of nearest settlement” (Areaqkm)**  
**C) standardized “Distance from lure stick location to the nearest single building” (LStDistEinzelBau).**

In the first steps of model building the influence of every single wildcat occurrence distance (WKin\*\*) was tested separately in the GLMM, including the year of data collection (Jahr) as random factor. The influence of every other scale variable (DistLStSiedl, Areaqkm, DistLStEinzelBau) was tested in distinct models for the distances 100 m and 500 m including the variable “Jahr” as random factor. Additionally, interaction between specific predictor variables was tested for wildcat records within distances of 100 m and 500 m (WKin100 and WKin500):

- “WKin100” \* “DistLStSiedl”
- “WKin100” \* “DistLStEinzelBau”
- “WKin500” \* “DistLStSiedl”
- “WKin500” \* “DistLStEinzelBau”
- “DistLStSiedl” \* “DistLStEinzelBau”
- “DistLStSiedl” \* “Areaqkm”
- “Areaqkm” \* “DistLStEinzelBau”



The GLMMs were built for every wildcat occurrence distance separately (WKin100 – WKin2000) including all other predictor variables listed above. Initially every GLMM was built with the linear term of each numeric predictor variable; subsequently the quadratic term of each numeric predictor variable was added to the model.

AIC values of the models were finally compared to figure out the qualitative best model for the given dataset, indicated by the lowest AIC value. R<sup>2</sup><sub>m</sub> and R<sup>2</sup><sub>c</sub> were calculated to show explained variance by the models for fixed and random factors.

To gain a more detailed insight into the influence of wildcat occurrences on domestic cat records at the lure stick a subset of the dataset was created containing only samples of "Domestic cat records at the lure stick" (HK = 1). Data was then split up into areas with or without wildcat records. A Wilcoxon rank sum test was chosen to investigate if domestic cats depart further away from the settlement if a wildcat was recorded within a maximum radius of 2000 m around the lure stick. As variables "Wildcat record within 2000 m to the lure stick" (WKin2000) and "Size of the nearest settlement" (DistLStSiedl) were chosen.

## 4 Results

### 4.1 Descriptive results

During the 8 years of lure stick based data collection (2008 - 2016) 1642 lure sticks were placed, encompassing 171 lure sticks with domestic cat records and 132 lure sticks with wildcat records. At 1081 lure sticks no hair samples could be collected and 258 of the collected samples could not be assigned to one of the *Felis silvestris* subspecies. In 2016 altogether 265 hair samples and 8 tissue samples could be collected and analyzed within the framework of this thesis. mtDNA and microsatellite fragment analysis resulted in 95 and 94 genetically proven European wildcat and Domestic cat records respectively. This entity of genetically assigned samples, represent a genotyping success rate of 71%.

Based on the ArcGIS buffer selection method, at 560 lure sticks a WK record within a radius of 2000 m could be estimated. Near 216 lure stick locations WK records within a distance of 100 m could be detected. Mean distance from lure stick location to the nearest settlement was 1065.7 m (min: 32.3 m; max: 3487.3 m). The mean distance from lure stick location to the nearest single building outside of settlement areas was 1595.63 m (min: 0.13 m; max: 4277.37 m). The largest settlement area close to lure stick locations (variable Areaqkm) was Freiburg im Breisgau.

### 4.2 Influence of every single predictor on domestic cat occurrence at lure sticks

Preliminary to model building the collinearity of predictor variables (Jahr, DistLStSiedl, Areaqkm, DistLStEinzelBau) was tested. The pairwise Pearson correlation test of collinearity between the predictor variables showed no correlation, as indicated by values between -0.198 and 0.377.

GLMMs were calculated with every single wildcat record distance to the lure stick (WKin\*\*; \*\* for the distances 100 m – 2000 m) in a distinct model (Table 4). Every model included the variable “Year of lure stick data collection” as random factor. The results displayed a significantly positive influence of WK occurrence on probability of domestic cat (HK) records at the lure stick for the distances 100 m (model A:  $p = 0.00244$  \*\*), 500 m (model B:  $p = 0.0372$  \*) and 2000 m (model F:  $p = 0.0164$  \*).

**Table 4: GLMM of every single wildcat occurrence variable (WKin\*\*) separately, including the variable “Year of lure stick data collection” (Jahr) as random factor. WK occurrence has a significantly positive influence on HK records at the lure stick within the distances 100 m, 500 m and 2000 m (models A, B and F respectively).**

Model	Variable	Estimate	Std. Error	z value	Pr(> z )
<b>A</b>	WKin100	0.6896	0.2275	3.031	0.00244 **
<b>B</b>	WKin500	0.4331	0.2079	2.084	0.0372 *
<b>C</b>	WKin1000	0.2938	0.1992	1.475	0.14
<b>D</b>	WKin1200	0.2743	0.1973	1.390	0.165
<b>E</b>	WKin1500	0.3478	0.1934	1.799	0.072 .
<b>F</b>	WKin2000	0.4653	0.1938	2.400	0.0164 *

Signif. codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’ 1

The influence of every other predictor variable in the GLMM was tested separately for the WK occurrence distances of 100 m (Table 5; models G - I) and 500 m (Table 5; models J - L). Results showed once more significantly positive influences of WK occurrences on HK records within these distances to the lure stick (models G – L). In addition the distance to settlements (DistLStSiedl) had a clear influence on HK occurrences at the lure sticks (models G & J). The probability of HK records at a lure stick decreased significantly with increasing distance to the settlement (model G:  $p = 8.28e-11$ ; model J:  $p = 7.09e-11$ ). However, the variables "Size of the nearest settlement" (Areaqkm) and "Distance from lure stick location to the nearest single building" (DistLStEinzelBau) did not display any significant influence on the response variable (models H, K and I, L respectively).

**Table 5: GLMMs of every single predictor variable for the wildcat occurrence distances 100 m (WKin100) and 500 m (WKin500). For every model the variable "Year of lure stick data collection" was set as random factor. In addition to WK occurrences within the particular distances, the distance to settlement (DistLStSiedl) has a significant influence on HK records at the lure stick (models G & J).**

Model	Variables	Estimate	Std. Error	z value	Pr(> z )
<b>G</b>	WKin100	0.7405	0.2328	3.181	0.00147 **
	DistLStSiedl	-0.7341	0.1130	-6.495	8.28e-11 ***
<b>H</b>	WKin100	0.66310	0.22813	2.907	0.00365 **
	Areaqkm	-0.12811	0.08472	-1.512	0.13050
<b>I</b>	WKin100	0.66250	0.22803	2.905	0.00367 **
	DistLStEinzelBau	-0.14946	0.08863	-1.686	0.09171 .
<b>J</b>	WKin500	0.5076	0.2121	2.394	0.0167 *
	DistLStSiedl	-0.7351	0.1128	-6.519	7.09e-11 ***
<b>K</b>	WKin500	0.41374	0.20814	1.988	0.0468 *
	Areaqkm	-0.13512	0.08457	-1.598	0.1101
<b>L</b>	WKin500	0.40891	0.20818	1.964	0.0495 *
	DistLStEinzelBau	-0.15387	0.08839	-1.741	0.0817 .

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

### 4.3 Interaction between predictor variables in the GLMM

Interaction between predictor variables was tested for all scale variables and wildcat occurrence distances of 100 m and 500 m (see paragraph 3.8 Statistical analysis).

Between the scale variables in the GLMM only the variable combination of "Distance from lure stick location to the nearest settlement" (DistLStSiedl) \* "Size of the nearest settlement" (Areaqkm) showed a highly significant negative influence on domestic cat records at the lure stick (HK) (Table 6; model R:  $p = 0.000346$  \*\*\*). The other variable combinations did not show any significant influence on the response variable. A detailed table of the statistical results is given in Appendix 4.

**Table 6: GLMMs of interaction between predictor variables and with WK records within a particular distance to the lure stick for the distances of 100 m (WKin100) and 500 m (WKin500). The variable interaction of "Distance from lure stick location to the nearest settlement" (DistLStSiedl) \* "Size of the nearest settlement" (Areaqkm) displays a significant influence on domestic cat records at the lure stick (HK).**

Model	Variable combinations	Pr(> z )
M	WKin100 * DistLStSiedl	0.77633
N	WKin100 * DistLStEinzelBau	0.1275
O	WKin500 * DistLStSiedl	0.8323
P	WKin500 * DistLStEinzelBau	0.186
Q	DistLStSiedl * DistLStEinzelBau	0.083 .
R	DistLStSiedl * Areaqkm	0.000346 ***
S	Areaqkm * DistLStEinzelBau	0.5050

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

#### 4.4 GLMMs with linear and quadratic terms

The GLMMs for every single wildcat record within a particular distance to the lure stick (WKin\*\*) were initially run with linear terms of all predictor variables. Results of these models showed a significantly positive influence of WK occurrence at distances of 100 m, 500 m and 2000 m and a highly significant negative influence of the variable "Distance from lure stick location to the nearest settlement" (DistLStSiedl) (Table 7). The lowest p-value ( $p = 0.00245$  \*\*) for WK occurrence was calculated for the distance of 100 m (WKin100), indicating a stronger influence at a small scale in the proximity of the lure stick (model HK1).

The clear negative influence of the variable "Distance from lure stick location to the nearest settlement" (DistLStSiedl) in the full models confirmed once more that probability of domestic cat records at lure sticks decreased with increasing distance to settlements and therefore underpinned the assumption that spatial distribution and activity patterns of domestic cats are linked to settlements (Table 7). However, the size of the nearest settlement (Areaqkm) and the distance from lure stick location to the nearest single building outside of settlement areas (DistLStEinzelBau) did not have any significant influence on the occurrence of domestic cats at lure sticks.

**Table 7: p-values of full models with linear terms of all predictor variables for each buffer distance separately. The models HK1, HK2 and HK6 display significant results for the variables WKin100, WKin500 and WKin2000 respectively. Additionally, the distance from lure stick location to the nearest settlement (DistLStSiedl) has significant influence on HK records at lure sticks.**

Model		WKin**	DistLStSiedl	Areaqkm	DistLStEinzelBau
HK1	WKin100	0.00245 **	1.49e-10 ***	0.10526	0.10526
HK2	WKin500	0.0231 *	1.36e-10 ***	0.0857 .	0.6847
HK3	WKin1000	0.0915 .	1.42e-10 ***	0.0830 .	0.6607
HK4	WKin1200	0.1350	1.66e-10 ***	0.0819 .	0.6861
HK5	WKin1500	0.0723	1.62e-10 ***	0.0866 .	0.6424
HK6	WKin2000	0.0263 *	1.77e-10 ***	0.0971 .	0.5933

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

Creating the models with addition of the quadratic term for every numeric predictor changed results especially for the variable "Size of the nearest settlement" ( $\text{Areaqkm}^2$  / Table 8; HK1.1 – HK6.1).

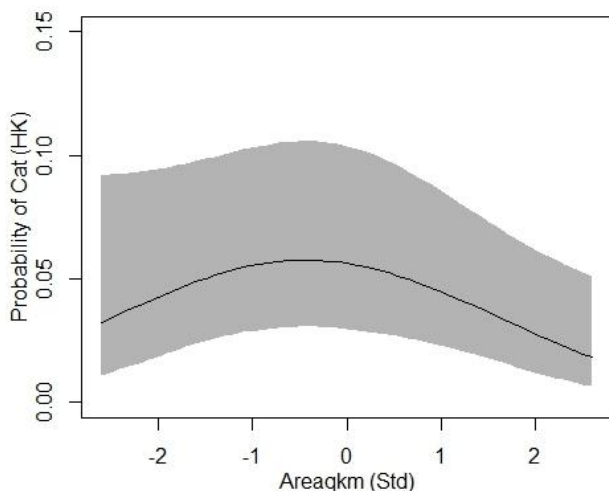
**Table 8: p-values of GLMMs with predictor variables in linear and quadratic terms for each WK occurrence distance (100 m – 2000 m).**

Model	WKin100	DistLSt Siedl	DistLSt Siedl <sup>2</sup>	Areaqkm	Area qkm <sup>2</sup>	DistLSt EinzelBau	DistLSt EinzelBau <sup>2</sup>
HK1.1	0.00265 **	4.42e-12 ***	0.02207 *	0.28066	0.03102 *	0.94734	0.18222
	<b>WKin500</b>						
HK2.1	0.0264 *	3.89e-12 ***	0.0234 *	0.2401	0.0316 *	0.9717	0.1822
	<b>WKin1000</b>						
HK3.1	0.1197	4.34e-12 ***	0.0260 *	0.2356	0.0329 *	0.9452	0.1808
	<b>WKin1200</b>						
HK4.1	0.1778	5.3e-12 ***	0.0267 *	0.2337	0.0323 *	0.9753	0.1813
	<b>WKin1500</b>						
HK5.1	0.0981 .	4.79e-12 ***	0.0247 *	0.2409	0.0361 *	0.9274	0.1768
	<b>WKin2000</b>						
HK6.1	0.0405 *	4.94e-12 ***	0.0240 *	0.2504	0.0409 *	0.8722	0.1783

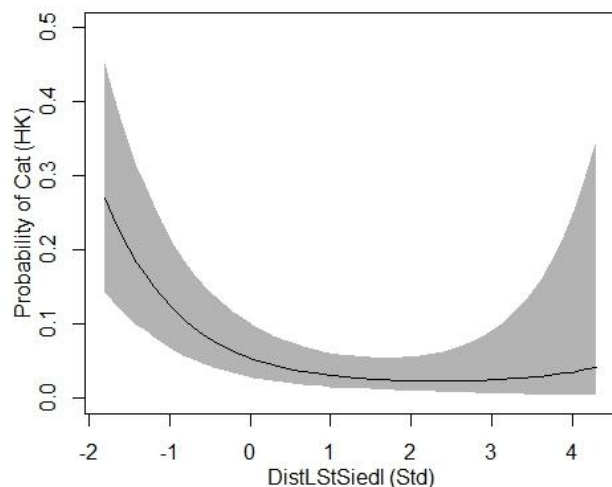
Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

In its quadratic term this variable had a significant influence on domestic cat occurrences at the lure stick. This result indicated that the probability of a domestic cat record at the lure stick increased with growing size of the settlement until settlement reached an intermediate size (Figure 9). In the surroundings of large settlements domestic cat occurrences at lure sticks were low again.

The variable "Distance from lure stick location to the nearest settlement" (DistLStSiedl) had in its quadratic term lesser but still significant effects on domestic cat records at lure sticks (Table 8). The probability of a domestic cat record at lure sticks decreased significantly with increasing distance to settlements, as already displayed by the linear term in the model (Figure 9).



**Figure 8: Probability of domestic cat occurrences at the lure stick (HK) concerning the size of the nearest settlement ( $\text{Areaqkm}^2$ ). Domestic cat records at the lure stick increase with size of the nearest settlement until an intermediate settlement size (model HK1.1).**



**Figure 9: Probability of domestic cat occurrences at the lure stick (HK) concerning the distance to the nearest settlement ( $\text{DistLStSiedl}^2$ ). Domestic cat records at lure stick decrease with increasing distance to the settlement area (model HK1.1).**

## 4.5 Likelihood of model prediction (AIC) and explained variance

AIC values for each model were compared to give indication on model quality for the given dataset. Models with all predictor variables including the quadratic term showed a slightly lower AIC value than models with linear terms (Table 9). Exclusion of the non-significant variable "Distance from lure stick location to the nearest single building" (DistLStEinzelBau) did not enhance the AIC value considerably.

**Table 9: AIC values for GLMMs with linear and quadratic terms. The models HK1-HK6 were built for the distances WKin100-WKin2000 with linear terms; the models HK1.1-HK6.1 were built for the distances WKin100-WKin2000 including linear and quadratic terms.**

Model	df	AIC	Model	df	AIC
HK1	6	1002.8487	HK1.1	9	996.9995
HK2	6	1006.3630	HK2.1	9	1000.6163
HK3	6	1008.4995	HK3.1	9	1002.9542
HK4	6	1009.0878	HK4.1	9	1003.5373
HK5	6	1008.1112	HK5.1	9	1002.6335
HK6	6	1006.4128	HK6.1	9	1001.1758

Explained variance for fixed and random factors resulted in values between 0.12 - 0.27 for models with linear terms and values between 0.11 – 0.28 for models including the quadratic term. The best explained variance ( $R^2_c = 0.285$ ) by fixed and random factors was achieved by the full model HK1.1 for the WK occurrence distance of 100 m and other predictor variables in its linear and quadratic terms (Table 10).

**Table 10: Result of the best fitting GLMM HK1.1 comprising the predictor variables in linear and quadratic terms (WKin100; DistLStSiedl; DistLStSiedl<sup>2</sup>; Areaqkm; Areaqkm<sup>2</sup>; DistLStEinzelBau; DistLStEinzelBau<sup>2</sup>)**

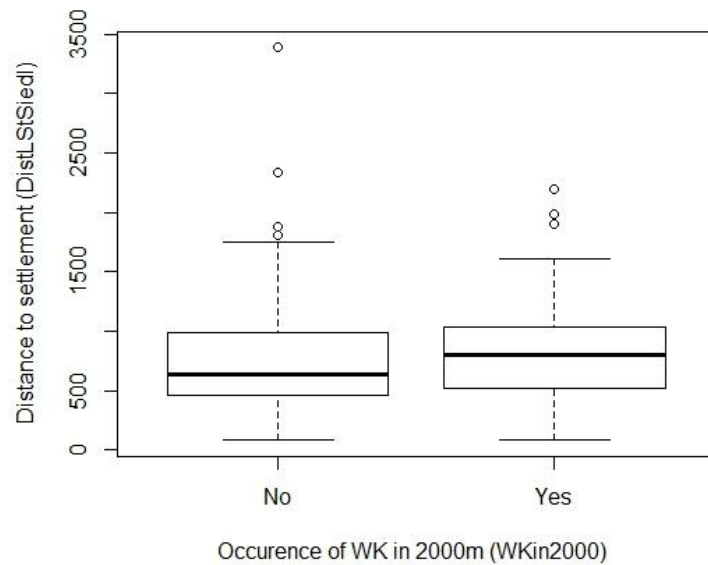
Model	Variables	Estimate	Std. Error	z value	Pr(> z )
HK1.1	(Intercept)	-2.942084	0.347086	-8.477	< 2e-16 ***
	WKin100	0.715301	0.238331	3.001	0.00269 **
	DistLStSiedl	-0.746704	0.107729	-6.931	4.17e-12 ***
	I(DistLStSiedl <sup>2</sup> )	0.158205	0.068607	2.306	0.02111 *
	Areaqkm	-0.101922	0.096735	-1.054	0.29206
	I(Areaqkm <sup>2</sup> )	-0.129665	0.060971	-2.127	0.03345 *
	DistLStEinzelBau	0.006276	0.092835	0.068	0.94610
	I(DistLStEinzelBau <sup>2</sup> )	0.091266	0.068590	1.331	0.18332

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

## 4.6 Influence of wildcat presence on domestic cat displacement from settlements

Since the variable "WK record within a distance of 2000 m to the lure stick" (WKin2000) had a significant effect in the models, this influence based on the variable "Distance to nearest settlement" (DistLStSiedl) for domestic cat occurrence at the lure stick was tested. Distances of domestic cat records at lure sticks to the nearest settlement areas ranged from minimum 80.9 m to maximum 3387 m (mean distance: 781.1 m). Although the distance of domestic cat records to settlements seemed to be slightly higher in areas where WK occurred (Figure 10), this result could not be confirmed by the Wilcoxon rank sum test. The non-significant p-value ( $p = 0.3603$ ) pointed out that domestic cats did not depart significantly further away from the settlement area if a wildcat record was located within a

maximum radius of 2000 m around the lure stick. This result also underpinned the non-significant interaction between the variables “WK record within a particular distance to the lure stick” and “Distance from lure stick location to the nearest settlement”. As pointed out by the GLMMs, the positive influence of wildcats on domestic cat records at lure sticks rather occurred on a small scale in near proximity to the lure stick.



**Figure 10: Boxplot of the distance of lure sticks with domestic cat records to the nearest settlement (DistLStSiedl) in areas with or without WK occurrences within a radius of 2000 m to the lure stick (WKin2000). Displacement distance of domestic cats from settlement areas is not influenced significantly by wildcat occurrence within a radius of 2000 m.**

## 5 Discussion

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The main goal of this study was to gain insight into domestic cat (*F.s. catus*) occurrence in forest habitats if the European wildcat (*F.s. silvestris*) was present in the particular area. Since the wildcat population in the Upper Rhine Valley in BW represents a fringe population expanding from France, this highly fragmented area with human settlements, anthropogenic landuse and home to lots of domestic cats was a suitable area to investigate occurrences of the two coexisting *Felis silvestris* subspecies. This mosaic of anthropogenic used landscapes is similar to other studies of wildcat and domestic cat interactions for instance in Hungary and Italy (Lecis et al. 2006). The study could reveal some underlying mechanisms of wildcat and domestic cat occurrences at lure sticks to gain insight into potential risk of hybridization.

### 5.1 Data sampling and laboratory analysis

Data sampling was conducted within the scope of the state wide wildcat monitoring program of FVA and BUND. Sampling areas were selected according to suspected wildcat occurrences in the particular area; therefore data was not evenly distributed throughout the county of BW. In some cases data collection was provided by several independent institutions in cooperation with FVA. Although data sampling was conducted by skilled staff according to a specified protocol, time spent per lure stick and precision of sample collection per individual lure stick can vary between persons and could have biased the sampling success. However, mtDNA and microsatellite analyses conducted within the scope of this thesis represented a genotyping success rate similar to other studies (Broquet et al. 2007; Steyer et al. 2012).

Assignment of samples to one of the *Felis silvestris* subspecies within this thesis was based on a combination of mtDNA and microsatellite results. Although recent studies often use microsatellite or SNP data for genotyping wildcat individuals (Devillard et al. 2014; Nussberger et al. 2014), for the setup of this thesis a categorization in either European wildcat or Domestic cat was considered to be adequate. This was assured by mtDNA and microsatellite genotyping. Even though no microsatellite data could be provided for earlier samples, assignment either to wildcat or domestic cat haplotypes on the basis of mtDNA was also reliable as shown by other studies (Randi et al. 2001; Steyer et al. 2016; Würstlin 2013). Inconclusive haplotypes, occurring in wild as well as domestic cat individuals, were excluded from further analysis. Even if hybrid individuals were not identified by this analysis technique, due to the low hybridization rate in the sampling area this error could be discounted.

### 5.2 Influence of wildcat occurrence on domestic cats

The results of the GLMMs showed that occurrence of wildcats in the proximity of the lure stick had a highly significant positive influence on domestic cats. This result stood in contrast to the first hypothesis of this thesis, assuming that proven occurrence of wildcats within their suitable habitat exclude occurrence of domestic cats. Different than expected, the results give indication that domestic cats might be attracted by wildcats if present in the vicinity of the particular lure stick. This might be explained by the phenomenon of sex pheromone excretion during the mating season.



Within the animal kingdom pheromones and olfactory signals are known to play a fundamental role in animal interaction. They act not only to distinguish each other as individuals, conspecific, predator or prey, amongst others, special sex pheromones operate to attract and find the right mating partner (Dulac & Torello 2003; Wyatt 2014). In cats, various studies on pheromones and odor show special flehmen behavior, mainly in males, and are suggested to play important roles in reproduction for both sexes (Bland 1979; Hart & Leedy 1987). Especially the pheromone precursor feline and the enzyme cauxin are species-, sex- and age dependent and provide species-specific information about the right mating partner (Miyazaki et al. 2006). In other species sex pheromones are clearly able to influence the behavior of their conspecifics as shown by studies on boar and hamsters. Special olfactory substances can directly affect oestrus in females and induce or facilitate copulatory behavior in males (Briand et al. 2004; Perry et al. 1980).

Studies on wildcat and domestic cat co-occurrences, for instance in the Bavarian Forest National Park, depicted broadly overlapping wildcat and domestic cat presences at camera traps indicating a spatial overlap of the two *Felis silvestris* subspecies (Beutel et al. 2017). Based on results of that study, spatial overlap was suspected to be a major threat to wildcats' genetic purity. In contrast to the broad forested areas of the Bavarian Forest National Park the area of the Upper Rhine Valley in BW is highly populated with scattered settlements, small fragmented forest patches and extensive agriculture. In this landscape domestic cats range frequently in direct neighborhood to forest patches and wildcat suitable habitat. Still, the relatively low hybridization rate of ~ 10% in the Upper Rhine Valley underlines, that spatial proximity alone is no explanation for a high hybridization rate (Streif et al. 2016). Therefore, some other underlying mechanisms might limit hybridization. One explanation could be the spatial organization of wildcat territories as detected by Beugin et al. (2016) in northwestern France. Female wildcats seem to be concentrated inside the forest while males having their territories at forest edges. This spatial structure could be a favorable condition for a low hybridization rate within the wildcat population, since female wildcats are expected to encounter less domestic cats. Telemetry studies on habitat utilization of wildcats in the Upper Rhine Valley showed the same spatial organization. Results depicted that open landscapes, such as meadows, agricultural fields and drainage channels are used almost exclusively by male wildcats (Streif et al. 2016). In addition, a recent telemetry study focusing on spatial organization of wildcats especially in an agriculturally dominated landscape pointed out a clear seasonal change in home range sizes for female and male wildcats. Whereas female home ranges were smallest during the mating season, male wildcats had their largest home ranges in spring, during the main mating season (Jerosch et al. 2017). This seasonal change in activity range sizes, especially in males, was also detected in other species such as Eurasian lynx (*Lynx lynx*) (Breitenmoser-Würsten et al. 2007). Comparable to findings in the Upper Rhine Valley female home ranges estimated by Jerosch et al. (2017) were notably smaller than home ranges of females in forested habitat but still included forest patches as much as possible. These results could also have important influences on encounter probability of wild and domestic cats and thus could minimize the potential risk of hybridization.

Within the close proximity of a lure stick small scale habitat structures might offer interesting requisites for wildcats as well as domestic cats and could additionally be an explanation for domestic cat occurrences at particular lure sticks. Since all lure sticks were placed within the frame of the national wide wildcat monitoring project, habitat of lure stick locations was selected according to wildcat's requirements or based on suspected wildcat occurrences. Numerous studies on wildcat habitat selection documented a high demand for cover and shelter (Beutel et al. 2017; Jerosch et al. 2010; Klar et al. 2008). Similar results were estimated by a study on habitat selection of domestic cats. Therein Metsers et al. (2010) revealed a clear avoidance of open areas with little cover and preference for covered habitat, being compatible to habitat selection of wildcats. Small scale habitat selection of other mammalian mesopredators, including the least weasel (*Mustela nivalis*), ermine stoat (*Mustela erminea*), polecat (*Putorius sp.*), martens (*Martes sp.*), Eurasian badger (*Meles meles*) and red fox (*Vulpes vulpes*) detected highly significant preferences of carnivores for forest/ farmland edges and a relationship between forest fragment size and probability of carnivore detection (Červinka et al. 2011). For domestic cats, included in that thesis, especially the distance to forest edges and size of the forest fragment showed significant influences on the detection of the species. Considering these results a closer look at the surrounding area of every lure stick could be reasonably including the aforementioned factors.

A closer look to influences of wildcats on domestic cat displacement from settlements pointed out that domestic cats do not displace further away from settlements if wildcats occurred in that particular area. This finding was underpinned by the non-significant results of variable interaction tested by the GLMMs M (WKin100 \* DistLStSiedl) and O (WKin500 \* DistLStSiedl) described in chapter 4.3. A study on factors influencing domestic cat home ranges detected a change in range size between diurnal and nocturnal activities but centers of home ranges did not shift between seasons (Kitts-Morgan et al. 2015). This might be an indication on domestic cat roaming patterns in general. However, direct influences of wildcats were not considered in that study. Besides, this result must be seen as a rough indication due to a small sample size. In contrast, other studies described long distance displacements by male domestic cats during the mating season in search for a female (Ferreira et al. 2011; Germain et al. 2008). This phenomenon is not only detected in domestic cats but also in wildcats (Jerosch et al. 2017; Streif et al. 2016), assuming that the two subspecies could also encounter outside of their common inhabited ranges. The phenomenon of hybridization of two subspecies in search of the right mating partner is already reported in other studies, for instance between Canada lynx (*Lynx canadensis*) and Bobcat (*Lynx rufus*) (Homyack et al. 2008; Schwartz et al. 2004). Due to lack of mating partners at its distribution fringe the threatened Canada lynx seem to reproduce with the more abundant Bobcat and produce fertile offspring. Hybridization due to habitat loss and habitat fragmentation along with the lack of mating partners of the same species is also a known problem in owl. Threatened Northern spotted owl males (*Strix occidentalis*) were detected to mate with female Barred owls (*Strix varia*), producing hybrids and may induce a dramatic decline in spotted owl populations (Gutiérrez et al. 2007; Miller et al. 2017). Therefore the problem of hybridization at the

border of its distribution range as well as landscape fragmentation should also be considered in European wildcat conservation.

### 5.3 Influence of settlements and anthropogenic landuse on domestic cats and their displacement

The distance from lure stick location to the nearest settlement (DistLStSiedl) showed a highly significant negative influence on domestic cat records at the lure stick, confirming the first part of the second hypothesis. The GLMM results proved that probability of domestic cat records at lure sticks decrease with increasing distance to settlement areas. This result fits into the picture of other studies on domestic cats and their ranging characteristics (Meek 2003; Metsers et al. 2010; Thomas et al. 2014). In the Bavarian Forest Beutel et al. (2017) stated, that “90% of domestic cat events at camera traps occurred within 1.1 km from human settlements”. Three-fourth of the lure sticks with domestic cat hair samples in this study were within a distance of 1005 m to the nearest settlement. A telemetry study of Barratt (1997) investigated home range sizes of suburban domestic cats as well as farm cats. Results showed that suburban domestic cats had a straight line displacement of up to 850 m with an extremely high variation in home range sizes. Compared to that, farm cats’ displacement was clearly lower and variation in straight line displacement was less. A somewhat smaller displacement distance of tracked domestic cats was estimated by the study of Thomas et al. (2014) who calculated a maximum daily ranging area of 656 m. Taking into consideration the densely populated area of the Upper Rhine Valley, in contrast to for instance large forested areas in the Bavarian Forest National Park or the Rothaargebirge (Beutel et al. 2017; Dietz et al. 2016), a domestic cat displacement of ~ 1 km might be enough to encounter wildcat individuals. A study on hybridization between African wildcats (*F. s. lybica*) and domestic cats (*F. s. catus*) in South Africa revealed a significant influence of distance to human settlements on the rate of hybridization of the two subspecies (Le Roux et al. 2015). Hybridization rate in that area declined clearly with increased distance to settlement areas.

The influence of the variable “Size of the nearest settlement” (Areaqkm) represented a significant influence on domestic cat occurrences at lure sticks only in its quadratic term. This result gave an indication that probability of domestic cat records increases with size of settlement until settlement areas reach an intermediate size. Beyond this value occurrence of domestic cats at lure sticks is declining, suggesting that domestic cats inhabiting large settlements do rather roam within settlement area boundaries. This goes in line with findings of Flockhart et al. (2016) who analyzed domestic cat abundances in a large city of 120,000 inhabitants with a settlement size of 86.7 km<sup>2</sup>. Results depicted a high density of free ranging domestic cats especially in residential areas and a clear decrease in abundance closer to woods and forested areas. A study conducted by Thomas et al. (2014) investigated ranging characteristics and habitat use of domestic cats in a settlement similar to Freiburg im Breisgau, being the largest settlement in this study. Mean daily ranges of domestic cats in their study were 1.94 ha, being similar to ranging areas of other studies (Barratt 1997; Meek 2003; Metsers et al. 2010). Although the GPS tracked individuals significantly avoided pure urban habitats, they displayed a preference for garden and other types of green habitat within the settlement area. This positive correlation of domestic cat detection in the closer proximity to parks and green areas

within the settlement area was also found by Flockhart et al. (2016). Similar to this Barratt (1997) pointed out that although domestic cats made excursions outside of the settlement area they spent the majority of time within the suburb boundaries. Hence, considering the expected daily ranging area of domestic cats being  $\sim 2$  ha, it can be predicted that free ranging domestic cats in larger cities rather use green habitats such as garden and parks within the settlement area boundaries than leave the settlement area. These findings were congruent with predicted results of variable influences in this study. Metsers et al. (2010) observed that free ranging domestic cats seem to have larger home ranges adjacent to open areas, compared to domestic cats in larger human settlements. This finding could additionally explain higher domestic cat records at lure sticks in the proximity of smaller villages. Moreover it can be expected that smaller villages are surrounded by less barriers for domestic cats such as roads or industrial areas.

The interaction of both variables "Distance from lure stick location to the nearest settlement" (DistLStSiedl) and "Size of the nearest settlement" (Areaqkm) showed a negative influence on domestic cat records in the GLMMs. This means that at the same distances to settlements occurrence of domestic cats at the lure stick is more likely in proximity of smaller settlements than of larger settlements. This result underlines the outcome of the quadratic term of the variable "Size of the nearest settlement" (Areaqkm) and goes in line with findings of other studies in the aforementioned paragraph.

Others than expected, the variable "Distance from lure stick location to the nearest single building" (DistLStEinzelBau) did not have any significant influence on domestic cat records in this study. One explanation for the non-significant influence of the distance to the nearest single building could be considering the category selection. Provided landscape data of single buildings outside of settlement areas were made according to a previous study (Ehrhart 2015) and included also cemeteries, camping areas, garden plots and holiday residences. A more specific categorization including only single farms could alter the significant influence of these results. Other studies in France and Spain detected a noteworthy linkage of domestic cat home ranges to the farms they inhabit (Barratt 1997; Ferreira et al. 2011; Germain et al. 2008).

## 5.4 Potential risk of hybridization

Nowadays the topic of hybridization of the European wildcat (*F.s. silvestris*) with its domestic counterpart the Domestic cat (*F.s. catus*) is an important issue in wildcat research, conservation and management. In Germany the European wildcat is recently expanding as shown by different studies based on genetic analyses (Steyer et al. 2016; Würstlin et al. 2016). Whereas some populations of the European wildcat depict extremely high rates of domestic cat introgression and the genetic extinction of regional populations (Beaumont et al. 2001; Pierpaoli et al. 2003) the German wildcat population still denote a very low hybridization rate of  $\sim 3.9\%$  (Steyer et al. 2016).

Although the wildcat population in the Upper Rhine Valley represents a fringe population expanding to the east and individuals roam in a highly fragmented area with lot of anthropogenic used landscapes and many free roaming domestic cats, the relative low hybridization rate ( $\sim 10\%$ ) still denote a

genetically healthy population (Streif et al. 2016; Würstlin et al. 2016). However, as detected in studies on Canada lynx and Northern spotted owl expansion of home ranges during mating seasons and fragmented habitat can lead to hybridization if there is a lack of mating partners of the same species (Miller et al. 2017; Schwartz et al. 2004). These circumstances might also be a potential risk of hybridization in the Upper Rhine Valley. To be able to assess the potential risk of hybridization in detail factors of influence from a small to a larger scale should be considered. On a small scale of investigation for instance intensified pheromone excretion during the mating season might be a driver for the attraction of the particular species in the near proximity to each other. In addition, habitat requisites favored by both subspecies and special microhabitat structures in the proximity can favor occurrences at a particular spot. On a larger scale individual home range sizes and spatial organization of territories should be taken into consideration. As confirmed by this and other studies distances to anthropogenic settlements had a significant influence on encounter of the species and suspected hybridization that should not be underestimated. In general human activities and species extinction due to hybridization are linked together, as revealed by a comparative review study (Todesco et al. 2016). As displayed in different species, the risk of hybridization should not be underestimated and management implications to enhance habitat conditions for the European wildcat are important to be continued.

## 6 Conclusion and Outlook

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Overall this thesis could give a first basic insight into important factors influencing domestic cat occurrences in wildcat suitable habitat. Although activity ranges of wildcats and domestic cats are expected to broadly overlap in the study area, hybridization is still at a relatively low level. The entire dataset of 10 years of wildcat lure stick monitoring revealed that the successfully expanding fringe population of wildcats is not hampered by the fragmented and densely human populated area of the Upper Rhine Valley. However, domestic cats and wildcats are expected to interact and encounter frequently, but underlying mechanisms seem to restrict successful hybridization.

The main result of this thesis was that wildcat occurrences in the area seem to attract domestic cats within the close proximity to the lure stick, rather than restrain them from entering wildcat suitable habitat. Reasons therefore can be suspected due to the mating season or intersexual displacement differences between males and females. Also some other habitat requisites being interesting for wild as well as domestic cats, such as ecotones, distances to forest edges and size of forest fragments can have been influencing this outcome. To gain more detailed information on this results detailed genetic analysis considering the sex of the individual as well as small scale habitat analysis at the particular lure spots might be reasonable. As expected, landscape related variables linked to anthropogenic used areas were a main influencing factor for domestic cat occurrences at lure sticks. However, a more specific categorization of single buildings outside of settlement areas could give a clearer view on influences of single farms on domestic cat displacement.

Besides settlement areas, the influence of roads on interaction of wild and domestic cats should be considered in further analysis. Studies on wild and domestic cat abundances, including habitat modeling, defined roads to be an important influencing factor (Flockhart et al. 2016; Klar et al. 2008). Finally, other important influencing factors such as weather conditions might be able to enhance the explained variance of the statistic models. Different studies on wildcats as well as domestic cats revealed weather conditions, such as snow cover and temperature, to have a notably influence on their presences (Beutel et al. 2017; Germain et al. 2008). These findings give suggestion for further analysis including weather conditions as important influencing factor for interaction between wildcats and their domestic counterparts.

To gain a deeper insight into the precise risk of hybridization between the European wildcat and the Domestic cat in BW further analysis might be recommendable. In particular data on different scales and including the factors mentioned above can facilitate to enhance management implications for the conservation of this elusive carnivore.

## 7 References

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## 8 Appendices

### *Appendix 1*

#### **Cooperating institutions for lure stick data collection:**

Naturpark SFW (Naturpark Schwäbisch-Fränkischer Wald), Naturpark SH (Naturpark Stromberg-Heuchelberg), LAZBW-WSF (Landwirtschaftliches Zentrum Baden Württemberg- Wildforschungsstelle), BUND (Bund für Umwelt und Naturschutz Deutschland), the municipality Rust as well as private initiative, both supervised by FVA.

#### ***Number of placed lure sticks per institution and year of data collection:***

	2008	2009	2010	2011	2012	2013	2014	2015	2016
<b>FVA</b>	62	48	97	47	230	193	160	152	300
<b>Naturpark SFW</b>	0	0	0	0	0	0	0	0	14
<b>Naturpark Stromberg-Heuchelberg</b>	0	0	0	105	0	0	0	0	0
<b>Privat</b>	0	0	0	0	0	0	6	0	0
<b>Privat dann FVA</b>	0	0	0	0	0	0	15	0	0
<b>Stadt Rust und FVA</b>	0	0	0	0	0	0	0	26	0
<b>WFS</b>	70	0	118	0	0	0	0	0	0

### *Appendix 2*

#### **ATKIS landuse categories used in this thesis:**

Selection of categories defined as suitable wildcat habitat by Erhart (2015). Categories were adjusted to the spatial extent of this thesis.

#### ***1. Suitable vegetation areas outside of forest:***

ATKIS Objektart	ATKIS Objektname	ATKIS unterart_name	ATKIS merkmakl_1	ATKIS merkmakl_12 name	Auswahl-kriterium nach
43007	Unland/ Vegetations- freie Fläche	Öffentliche Zwecke			Objektname
43006	Sumpf				
44006	Stehendes Gewässer		BWS (Bewuchs)	Gebüsch	Merkmal_12 name
43001	Landwirt- schaft	Baumschule  Grünland	BWS (Bewuchs)	Gebüsch Gehölz	Definition Sandrini  Merkmal_12 name
43005	Moor		BWS (Bewuchs)		Objektname
42001	Straßen- verkehr	Verkehrs- begleitfläche	BWS (Bewuchs)	Gebüsche Gehölz	Merkmal_12

				Baumbestand, Laubholz Baumbestand, Laub- und Nadelholz	
43003	Gehölze		BWS (Bewuchs)	Gebüsch Baumbestand, Laubholz	Objektname
41008	Sport-, Freizeit-, Erholungs- fläche	Freizeitanlage	BWS (Bewuchs)	Baumbestand, Laub- und Nadelholz	Waldkletter- garten

**2. Meadows, farmland and areas with suitable coverage:**

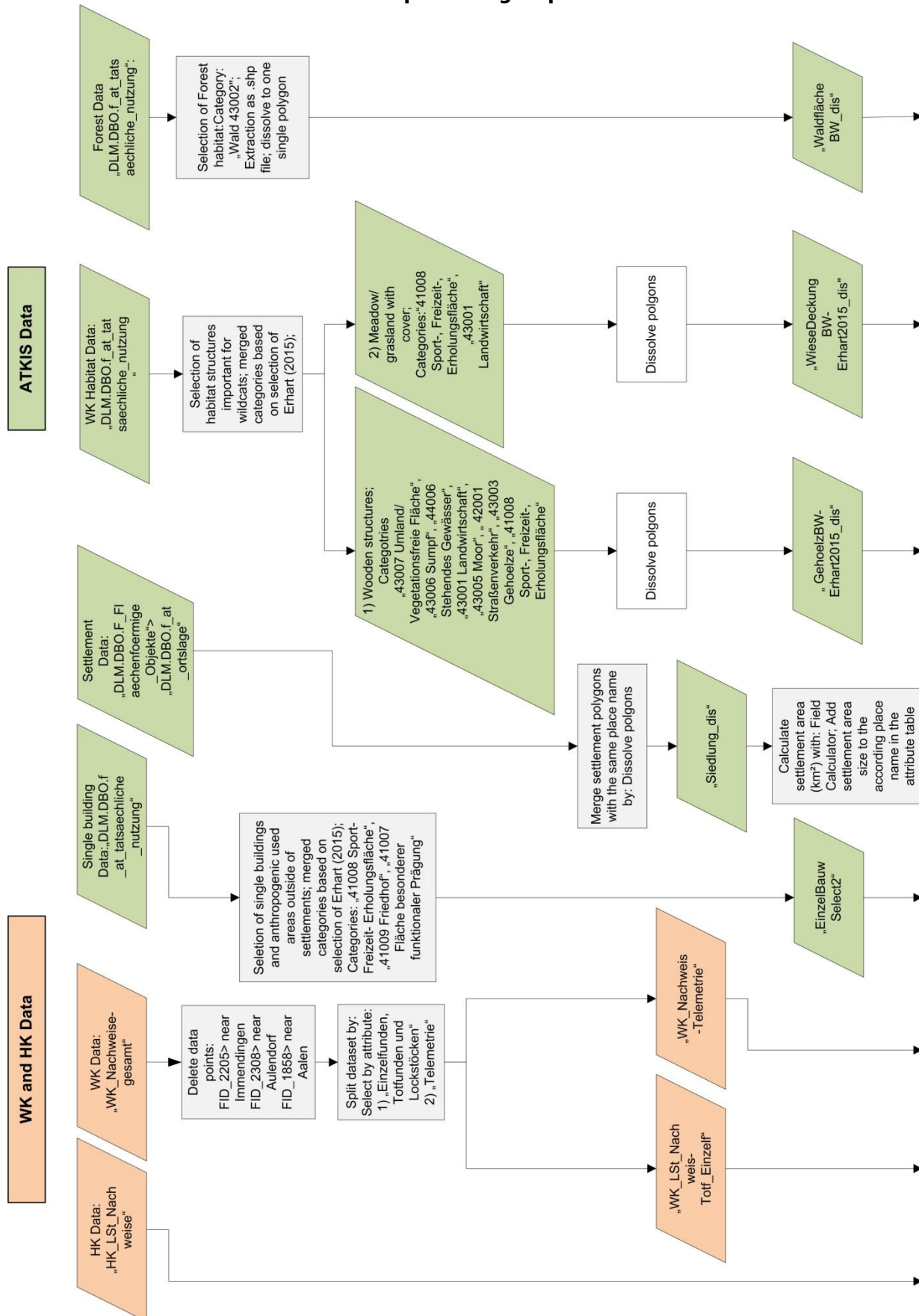
ATKIS Objektart	ATKIS Objektname	ATKIS unterart_name	ATKIS merkmal_1	ATKIS merkmal_12 name	Auswahl- kriterium nach
41008	Sport-, Freizeit-, Erholungs- fläche	Gebäude- und Freifläche Erholung	BWS (Bewuchs)	Baumbestand, Laubholz	Merkmal_12
43001	Landwirt- schaft	Grünland Obstplantage Streuobstwiese Weingarten	BWS (Bewuchs)  BWS (Bewuchs)	Baumbestand, Laubholz  Baumbestand, Laubholz	Merkmal_12 unterart_n unterart_n unterart_n

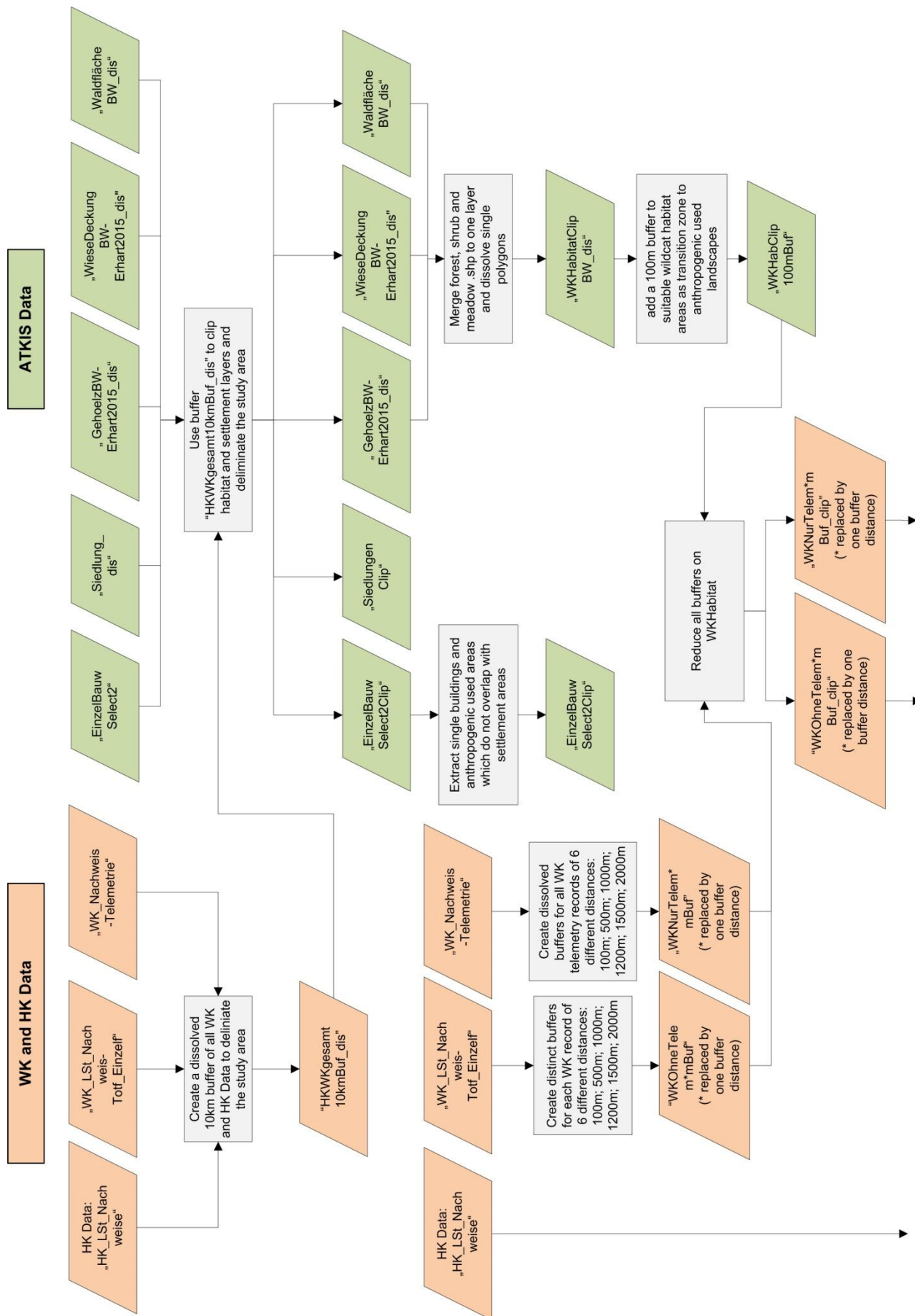
**3. Anthropogenic landuse and single buildings outside of settlement areas:**

ATKIS Objektart	ATKIS Objektname	ATKIS unterart_name	ATKIS merkmal_1	ATKIS merkmal_12 name	Auswahl- kriterium nach
41008	Sport-, Freizeit-, Erholungs- fläche	Campingplatz Freizeitanlage Freilicht- museum Freilichttheater Gebäude- und Freifläche Erholung Schrebergarten Wochenend- und Ferienhaus- fläche			unterart_n eigenname eigenname  eigenname unterart_n  unterart_n  unterart_n
41009	Friedhof				Objektname
41007	Fläche besonderer funktionaler Prägung	Offen			Objektname & eigenname

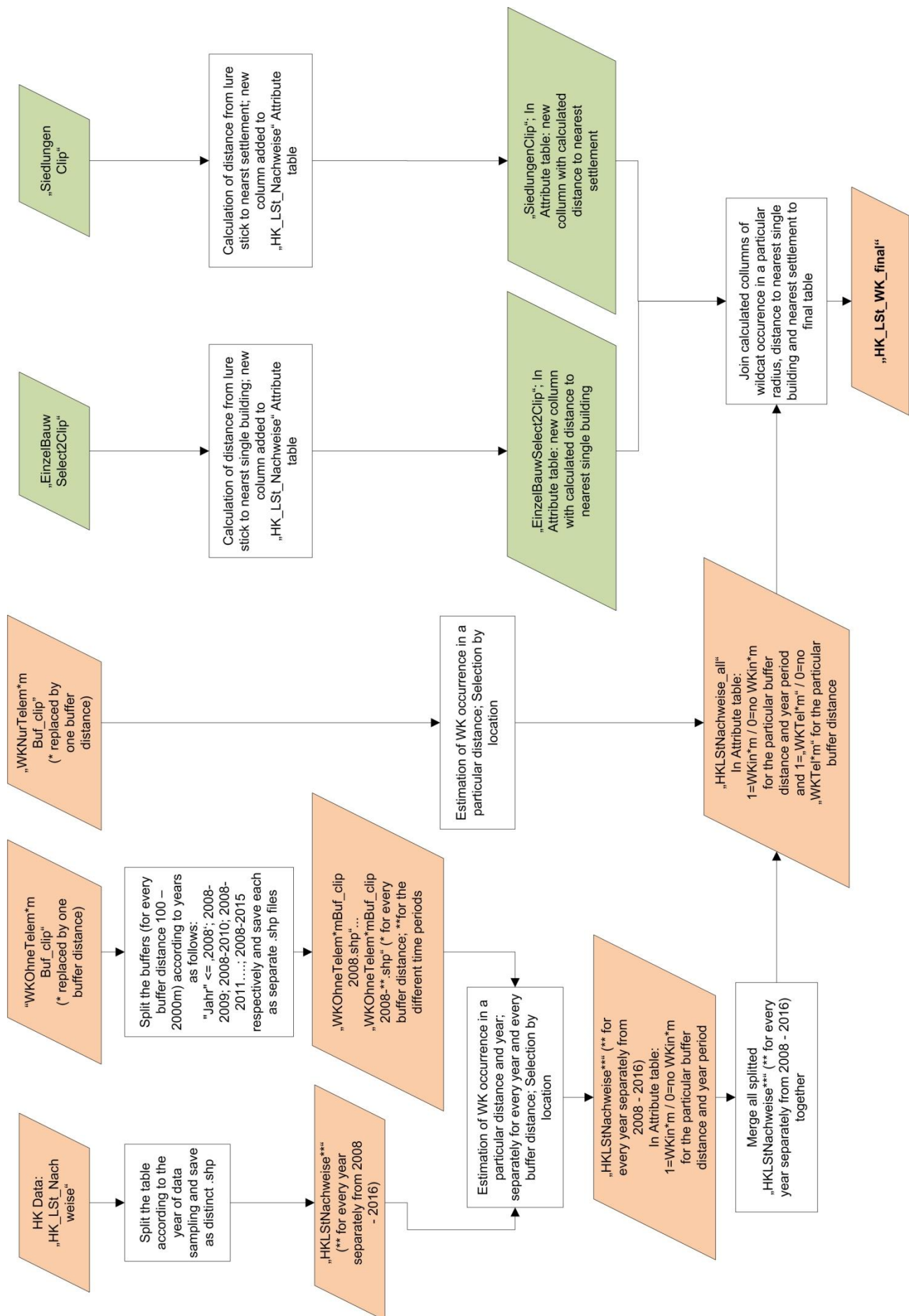
## Appendix 3

Detailed flow chart of the Arc GIS processing steps for creation and selection of variables:











*Appendix 4***Detailed results of interaction test between variables in the GLMMs:**

Model	Variables	Estimate	Std. Error	z value	Pr(> z )
<b>M</b>	<b>WKin100 *</b> <b>DistLStSiedl</b>	0.08092	0.28482	0.284	0.77633
<b>N</b>	<b>WKin100 *</b> <b>DistLStEinzelBau</b>	-0.42238	0.27716	-1.524	0.1275
<b>O</b>	<b>WKin500 *</b> <b>DistLStSiedl</b>	0.05415	0.25577	0.212	0.8323
<b>P</b>	<b>WKin500 *</b> <b>DistLStEinzelBau</b>	-0.31295	0.23682	-1.321	0.186
<b>Q</b>	<b>DistLStSiedl *</b> <b>DistLStEinzelBau</b>	0.16649	0.09604	1.734	0.083 .
<b>R</b>	<b>DistLStSiedl *</b> <b>Areaqkm</b>	-0.3755	0.1049	-3.578	0.000346 ***
<b>S</b>	<b>Areaqkm *</b> <b>DistLStEinzelBau</b>	-0.05579	0.08367	-0.667	0.5050

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1