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Influence of olfaction on the breeding performance of two passerine birds, the great tit (*Parus major*) and the blue tit (*Cyanistes caeruleus*)

Master Thesis

for obtaining the academic degree Master of Science
in Wildlife Ecology and Wildlife Management

Submitted by: Wiltrut Ute KOPPENSTEINER
Immatriculation number: 0840137

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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.

03-Feb-2017

Date

Signature

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Abstract

The role of olfaction in birds has long been underestimated by scientists, but its relevance is nowadays widely recognised, even for small passerine birds. However, the use of olfactory abilities especially in free-ranging bird populations remains poorly explored. Besides olfaction, visual cues like colours are important for birds, e.g. for nest material choice. In this study, I investigated in free-ranging nest box populations of great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) whether choice of nest material is odour and/ or colour dependent. Further, I studied whether the odour of lavender (*Lavendula angustifolia*) affected nestling condition, e.g. via effects on ectoparasites, and nest predation from snakes. Two independent experiments were conducted on the effects of a) lavender odour and colour on the choice of nest material and b) lavender odour on the nestling condition and nest predation.

My results showed that non-odour-manipulated and red wool was significantly chosen more often as nest material. On the nestling condition, the use of lavender odour showed no significant effects. However, lavender had significant effect on nest predation, especially regarding Aesculapian snakes (*Zamenis longissimus*). My results supported the hypothesis that great and blue tits have olfactory abilities, preferentially chose red colour as nest material and that lavender odour is effective as snake repellent in free-ranging populations of secondary hole-breeding birds.

Zusammenfassung

Der Geruchssinn spielt bekanntermaßen eine wichtige Rolle bei der Wahrnehmung der Umwelt und ist bei vielen Taxa sehr gut erforscht. Umso erstaunlicher ist, dass lange Zeit angenommen wurde, Vögel würden über keinen oder einen nur unbedeutenden Geruchssinn verfügen. Heute ist weitgehend anerkannt, dass auch Vögel eine gewisse Geruchsschärfe besitzen und, dass der Geruchssinn auch bei Passeriformes eine Rolle in verschiedenen biologischen Kontexten spielt. Dennoch ist nach wie vor wenig über die olfaktorische Wahrnehmung wildlebender Vogelpopulationen bekannt. Auch ist zu berücksichtigen, dass neben dem Geruchssinn die visuelle Wahrnehmung von Vögeln eine entscheidende Rolle spielen kann, etwa bei der Wahl des passenden Nistmaterials.

In dieser Studie untersuchte ich anhand wildlebender Kohlmeisen (*Parus major*) und Blaumeisen (*Cyanistes caeruleus*), ob Nistmaterial anhand von Geruch und/ oder Farbe des Materials gewählt wird. Ich ging auch der Frage nach, ob Lavendel (*Lavendula angustifolia*) einen Effekt auf die Kondition der Nestlinge, etwa durch die Reduktion von Ektoparasiten, und auf die Nestprädation, etwa durch eine mögliche Wirkung als Schlangenabwehr hat.

Die Ergebnisse zeigen klar, dass geruchlose und rote Wolle signifikant öfter als Nistmaterial gewählt wurde. Der Versuch die Kondition der Nestlinge betreffend, brachte kein signifikantes Ergebnis. Aber Lavendel zeigte einen signifikanten Effekt in der Nestprädation, da Nester mit Lavendelgeruch weniger ausgeraubt wurden. Dieser Effekt zeigt sich besonders im Hinblick der Äskulapnatter (*Zamenis longissimus*) als Nestprädator. Meine Ergebnisse weisen darauf hin, dass sowohl Kohl- als auch Blaumeisen eine gewisse Geruchsschärfe besitzen, rotes Nistmaterial bevorzugt wählen und Lavendel eine Wirkung als Schlangenabwehr hat.

Table of content

1.	Introduction	8
2.	Methods and Materials	13
2.1.	Study organism.....	13
2.2.	Experiment A - Odour and colour dependent nest material choice	14
2.2.1.	Study area	14
2.2.2.	Study design	14
2.2.3.	Statistical analysis	16
2.3.	Experiment B - Effects of odour on the breeding performance.....	17
2.3.1.	Study area	17
2.3.2.	Study design.....	18
2.3.3.	Statistical analysis	19
3.	Results.....	20
3.1.	Odour dependent choice of nest material.....	20
3.2.	Colour dependent choice of nest material.....	21
3.3.	Effect of odour on nestling condition	24
3.4.	Effect of odour on nest predation.....	27
4.	Discussion	28
4.1.	Odour dependent choice of nest material.....	28
4.2.	Colour dependent choice of nest material.....	29
4.3.	Effect of odour on nestling condition	31
4.4.	Effect of odour on nest predation.....	32
5.	References	34
6.	Appendices	39

List of figures

Figure 1: Set-up of the experiment on nest material choice.	15
Figure 2: Withdrawal quantities of the offered experimental wool	20
Figure 3: Average spectral reflectance of the three colours of the experimental wool	21
Figure 4: Withdrawal quantities of blue, green and red wool	22
Figure 5: Amount of retrieved coloured experimental wool of the nest boxes	23
Figure 6: Overview of the results of the nest dissection	23
Figure 7: Mean body mass of nestlings at day 10	26

List of tables

Table 1: Overview of the distribution of nest boxes included in the experiment on the effects of lavender odour on the breeding performance	24
Table 2: Overview of the distribution of nest boxes and number of nestlings included in the analyses of the influence of lavender on the nestling condition	25
Table 3: Differences in predation between areas, species and odour groups	27
Table 4: Differences in predation between odour groups regarding predation by Aesculapian snakes versus other predators	27

1. Introduction

Olfaction, or the sense of smell, is defined in terrestrial vertebrates as the chemosensory ability to obtain information about the environment from airborne, volatile chemical substances (Nef 1998; Ache and Young 2005). Thus, olfaction is responsible for the obtainment of odours and, besides vision and audition, an important sense for analysing the surrounding (Nef 1998). The importance of olfaction in different biological contexts has been investigated in many taxa, e.g. mammals and reptiles (e.g. Kenneth 1967; Nef 1998; Ache and Young 2005). However, the role of olfaction in birds has been underestimated for a long time and is still highly unrepresented in ornithologic studies, especially regarding Passeriformes (Kenneth 1967; Amo et al. 2008; Clark et al. 2015).

The importance of olfaction in birds has been debated for decades in the scientific society (e.g. Kenneth 1967; Mennerat et al. 2005; Balthazart and Taziaux 2009). It was believed for a long time that olfactory organs of birds and their abilities were minimal or lacking, ignoring anatomy studies that confirmed the existence of smelling organs in certain bird species (Bang 1960, 1971; Bang and Cobb 1968; Kenneth 1967; Hagelin and Jones 2007; Wenzel 2007). The first indications for avian olfaction focused on birds with large olfactory organs, like the brown kiwi (*Apteryx mantelli*), the turkey vulture (*Cathartes aura*), the trinidad oilbird (*Steatornis caripensis*), the laysan albatrosses (*Diomedea immutabilis*) and the black-footed albatrosses (*Diomedea nigripes*) (Bang 1960; Kenneth 1967). An important evidence in the research of the olfactory acuity in birds is the study by Bang (1960), in which the olfactory bulb-to-hemisphere-ratios of numerous bird species were measured. The olfactory bulb is the region of the brain that processes the odour input (Bang 1960, 1971; Bang and Cobb 1968). Based on the olfactory bulb-to-hemisphere-ratio, it was assumed that birds with a high ratio (e.g. turkey vulture) had higher olfactory acuity than those with a lower ratio (e.g. house sparrow (*Passer domestica*)) (Bang and Cobb 1968; Buitron and Nuechterlein 1985; Clark et al. 2015). Following studies demonstrated that the sense of smell is used in, e.g. food location of brown kiwi and turkey vulture, orientation and navigation of homing pigeons and seabirds and nest location by numerous Procellariiformes (reviewed in Balthazart and Taziaux 2009).

Nowadays, the relevance of olfaction in birds in different biological contexts is widely recognised, even for small passerines. Passeriformes like, e.g. brown-headed cowbirds (*Molothrus ater*) (Clark and Mason 1991), tree swallows (*Tachycineta bicolor*) and cedar

waxwings (*Bombycilla cedrorum*) (Clark 1991), great tits (*Parus major*) (Clark et al. 1993), zebra finches (*Taeniopygia guttata*) (Kelly and Marples 2004) and European starlings (*Sturnus vulgaris*) (Clark and Manson 1985; Clark 1990; Clark and Mason 1987) were demonstrated to discriminate different odours. The studied passerine birds use their sense of smell in, e.g. food location (Kelly and Marples 2004), nest recognition (Caspers and Krause 2011), mate recognition (Whittaker et al. 2010) and predator recognition (Roth et al. 2008; Amo et al. 2008; Amo et al. 2011).

Furthermore, olfaction plays a role in the protection of nestlings against ectoparasites in the nest of certain bird species. Ectoparasites can result in nestling mortality and nest desertion. Most responsible for nestling mortality are dipterans, fleas, ticks, and mites (Herman 1955 reviewed in Wimberg 1984). These ectoparasites are most often found in nests of bird species reusing their nest sites (Wimberger 1984), like hole-breeding species (e.g. European starlings, great tits and blue tits) (e.g. Clark and Manson 1985). European starlings were demonstrated to use aromatic herbs which were presumed to have positive effects on their nestlings due to suppression of parasites and bacteria (e.g. Clark and Manson 1985; Clark and Mason 1987; Clark 1990; Gwinner et al. 2000). Certain aromatic herbs were experimentally investigated to its effects on the most common nest-dwelling ectoparasites and showed positive effects on the immune system of the nestlings (Gwinner et al. 2000) or a reduction in abundance of parasites (Shutler and Campbell 2007; Tomás et al. 2012). Based on these findings, hypotheses about the function of aromatic herbs in nests were proposed: i.e. the “nest protection hypothesis” describing that birds use herbs with chemical compounds that keep the nest free from parasites because the aromatic compounds might mask chemical cues used by ectoparasites to find hosts (e.g. Wimberger 1984; Clark and Manson 1985) and the “nest drug hypothesis” suggesting that certain herbs in the nest stimulate the immune system of the nestlings (Gwinner et al. 2000). However, the suggested positive effects of aromatic herbs on the nestling growth (measured with body mass) due to ectoparasite-repellence and immune system improvement was verified only in few studies (but see Gwinner et al. 2000; Gwinner and Berger 2006; Mennerat et al. 2009a).

Although the interest in the role of olfaction in passerines has increased during the last decades, the use of avian olfaction in natural conditions is still poorly explored (Lambrechts and Hossaert-McKey 2006), especially regarding its role in nest building and maintaining (Petit et al. 2002). In 2000, the first demonstration of free-ranging Corsican blue tits (*Cyanistes caeruleus obliastreae*) using their olfaction in the protection of nestlings with aromatic plants

was published (Lambrechts and Dos Santos 2000), followed by more studies about the role of aromatic herbs in Corsican blue tit nests in free-ranging populations (Petit et al. 2002; Pires et al. 2012). Among other herbs, Corsican blue tits incorporated lavender (*Lavendula spec.*) in their nests (Lambrechts and Dos Santos 2000; Petit et al. 2002; Pires et al. 2012) and refreshed the herbs after a removal experiment (Lambrechts and Dos Santos 2000). Further, Lambrechts and Dos Santos (2000) showed that Corsican blue tits used herbs as nest material which were not available in the direct surrounding of the nest sites, indicating that tits actively search specific herbs such as lavender. Until now, it has not been demonstrated if other tit species (e.g. great tits) also incorporate nest material with the odour of lavender.

The essential oils of lavender were demonstrated to have an antimicrobial activity against both bacteria and fungi (Adam et al. 1998; Hanamanthagouda et al. 2010). Lavender showed also a high repellent effect on blood-sucking insects in a laboratory experiment with domestic chicks (Lafuma et al. 2001). In addition, essential oils of lavender were demonstrated to have repellent effects on the brown treesnake (*Boiga irregularis*), resulting in aggressive behaviour when exposed to the odour (Clark and Shivik 2002).

Therefore, the odour of lavender might also be of interest in context with nest predation by snakes. Nest predation is a primary source of reproductive failure for passerine birds (Sperry et al. 2009; Klug et al. 2010; Cox et al. 2013; Quan and Li 2015). Mammals are reported to be frequent nest predators in grasslands, whereas snakes are more frequent predators in forests and shrublands and predate the nests more frequently during the nestling stage (Cox et al. 2013). In my study area, the Aesculapian snake (*Zamenis longissimus*) is a frequent nest predator (Winkler 2009; Sperandio 2012; Weiß 2013). The brown treesnake and the Aesculapian snake are both members of the family Colubridae, wherefore lavender might have similar effects on the Aesculapian snake as reported for the brown treesnake.

In the present study, the essential oils of lavender were used to determine whether great tits and blue tits choose nest material with the odour of lavender, as it was shown for European starlings (Clark and Manson 1985) and Corsican blue tits (Lambrechts and Dos Santos 2000). Further, possible effects of the odour of lavender were investigated on the nestling condition and nest predation in free-ranging populations of great and blue tits.

Beside olfaction, vision is an important sense to obtain information about the environment. Regarding visual cues that may affect the resource search in birds, it is important to consider that birds see the world in other ways than humans (e.g. Roper 1997). Most bird species are reported to use their vision to obtain information about their environment, and many birds are sensitive to a wider range of spectral colours including ultra-violet light (e.g. Hart 2001; Hill and McGraw 2006). Although, the role of avian vision is well studied in certain biological contexts, e.g. food recognition (Marples et al. 1998; Hotova Savadova et al. 2010; Schmidt et al. 2004), mate choice and reproduction behaviour (Mahr et al. 2012; Götmark and Ahlström 1997), little is known about the role of vision in nest material choice.

Examples of studies regarding colour preference in nest material choice are contradicting and often focus on captive-reared birds, i.e. zebra finches (*Taeniopygia guttata*). In the study by Sargent (1965), the zebra finches preferred brown over green nest material, and did not choose red material. In contrast, Muth and Healy (2011) showed an initially preference for green nest material and a change in their colour preference due to a successful breeding attempt with the un-preferred colour (brown) of nest material. In a subsequent study, zebra finches strongly preferred blue over yellow and red nest material (Muth et al. 2013). Although these studies showed that zebra finches choose their nest material dependent on the colour of the material, it is not clear what causes this colour preference of nest material (Muth et al. 2013; Muth and Healy 2011, 2014; Sargent 1965). Regarding free-ranging tits, observation on the nest material showed a frequent incorporation of various coloured wool in the nest (Schöll and Hille 2014; Surgey et al. 2012; Harrison et al. 2004). Surgey et al. (2012) showed a tendency that great tits use red and green wool more often than white and yellow, whereas blue tits used red and white wool more often than green or yellow wool as nest material. These differences in colour-dependent choice missed level of significant, however the reported tendency indicates that the choice of nest material in context with colour might be species-specific. It is still unclear if great and blue tits have a colour preference in choosing nest material. To verify the reported possible effect of colour on choosing nest material also by great and blue tits further studies are necessary. In the present study, nest material in three different colours were offered to investigate a possible colour-dependent choice of free-ranging great and blue tits.

Nests of great and blue tits consist of a high amount of green material (e.g. moss; Harrison et al. 2004; Bauer et al. 2005) and some passerines were shown to incorporate green aromatic herbs in their nests as discussed above. Thus, tits might choose green coloured nest

material because of an initially preference for green material. Further, the colour red is a common colour signal and plays a role in many biological contexts, e.g. food choice (e.g. Hotova Savadova et al. 2010; Gamberale-Stille et al. 2007; Marples et al. 1998) and parental investment in context with the red mouth colour of nestlings (Götmark and Ahlström 1997). Therefore, the birds might react to the red coloured nest material. Additionally, blue coloured nest material was offered because of the proven colour preference in zebra finches (Muth et al. 2013), the relevance of blue colour in mate choice in blue tits (e.g. Mahr et al. 2012) and the observation that blue tits decorate their nests with blue material (*personal observation*, 2014).

The aim of this study was to investigate whether the choice of nest material is odour and/or colour dependent and whether the specific odour of lavender has positive effects on the nestling condition and lowers the nest predation rate of free-ranging great and blue tits. Thus, to test for odour linked choice when collecting nest material and their consequences, I conducted two independent experiments. The first one (experiment A) focused on the choice of nest material and tested the following hypotheses:

- 1) Great tits and blue tits more often choose odour-manipulated nest material compared with non-manipulated one because olfaction plays a role in the choice of nest material as reported for European starlings (Clark and Manson 1985) and Corsican blue tits (Lambrechts and Dos Santos 2000).

- 2) The colour of the nest material affects its choice because also vision plays a role in the choice of nest material as reported for zebra finches (e.g. Muth et al. 2013).

The second experiment (experiment B) focused on the possible effects of lavender on the breeding performance and tested the following hypotheses:

- 3) The body mass of the nestlings at day 10 is higher in nests manipulated with lavender than in nests without odour-manipulation because lavender prevents the nestlings from parasites and bacteria and strengthens the nestling condition based on the “nest protection hypothesis” (e.g. Wimberger 1984) and “nest drug hypothesis” (Gwinner et al. 2000).

- 4) Nests without the odour of lavender are more likely to be predated than nests with odour-manipulated nest materials because lavender odour has a predator-repellent effect, especially regarding snakes (Clark and Shivik 2002).

2. Methods and Materials

2.1. Study organism

The great tit (*Parus major*) and the blue tit (*Cyanistes caeruleus*) are common breeding birds in Central Europe and occur in a diversity of habitats such as deciduous woods, montane regions and urban areas. Great tits exhibit on average 14 cm body length and 17.6 - 18.9 g body mass (♀ - ♂), blue tits are on average smaller with 11.5 cm body length and 11.0 - 11.5 g body mass (♀ - ♂) (Bauer et al. 2005). Great tits and blue tits are secondary hole-breeders which built their nests in different holes in woods, parks and gardens and accept nest boxes as breeding sites (Bauer et al. 2005; Cramp and Perrins 1993). Within these holes, the great and the blue tits built their nests, which consists of diverse materials such as moss, grass interspersed with feathers, wool and animal origin hair (Harrison et al. 2004). In general, populations of great and blue tits are most affected by food limitation during winter, optimal nest sites and predation (e.g. Cramp and Perrins 1993; Bauer et al. 2005).

In Vienna, Austria, great tits are the second most frequent breeding passerines and occurred throughout the city (Wichmann 2009). About 25 % of great tits breed in forest habitats like the Wienerwald (Wichmann 2009). In Central Europe, the breeding season of great tits starts in April, occasionally earlier due to higher temperature and good weather condition, i.e. with end of February to begin of March (Bauer et al. 2005). The clutch size normally ranges between 6 - 13 (up to 15) eggs (Bauer et al. 2005). The incubation starts with full clutch and requires normally 13 - 14 days (Harrison et al. 2004). During this stage, the female great tit is fed by the male. The nestling time lasts 18 - 21 days (Harrison et al. 2004). Great tits have one to two broods which is mostly influenced by food availability (Bauer et al. 2005).

The blue tit is also a common species in Vienna, but does not occur as frequent as the great tit throughout the city due to assumed nest site competition with great tits (Wichmann 2009). In Vienna, blue tits have the highest abundance in oak rich woods and riparian forests (Wichmann 2009). In Central Europe, blue tits begin to breed in mid-April until early in May, occasionally also with end of March to begin of April (Bauer et al. 2005). The clutch size of blue tits normally ranges between 6 - 14 (up to 16) eggs (Bauer et al.). The incubation period starts with the second last egg and requires 13 - 15 days. During this stage, the male blue tit feeds the female. The nestling stage lasts 18 - 21 days (Harrison et al. 2004). Blue tits normally have only one brood per year (Bauer et al. 2005).

2.2. Experiment A - Odour and colour dependent nest material choice

2.2.1. Study area

The experiment took place at the Wilhelminenberg (48.219983° N, 16.282982 ° E) which is part of the Wienerwald of Vienna, Austria. The study area was situated in the forests of the “Forschungsinstitut für Wildtierforschung” (FIWI) and had an area of about 6.4 ha. The area is mainly dominated by European hornbeams (*Carpinus betulus*), oak trees (*Quercus spec.*) and beech trees (*Fagus sylvatica*). In 2009, a total of 44 wooden nest boxes of six different types were fixed on tree trunks by the “Konrad-Lorenz-Institut für Vergleichende Verhaltensforschung” (KLIVV). Nest boxes of the types “blue tit” (120 x 155 x 250 mm, entrance 26 mm), “long-old” (180 x 160 x 480 mm, entrance 35 mm), “long-new” (180 x 185 x 470 mm, entrance 35 mm), “short-old” (165 x 170 x 245 mm, entrance 32 mm), “short-new” (180 x 185 x 240 mm, entrance 32 mm) and “Urwald” (150 x 160 x 470 mm, entrance 45 mm) were installed at the FIWI forest (for map see Appendix 1). In addition, a total of 18 nest boxes which were installed at the area of the KLIVV (area about 2.8 ha) in 2004 were included in the experiment. These 18 nest boxes consisted of various types: eight “blue tit” (120 x 155 x 250 mm, entrance 26 mm), one “long-old” (180 x 185 x 470 mm, entrance 35 mm), two “short-old” (165 x 170 x 245 mm, entrance 32 mm), four “Schwegler - great tit”, one “Schwegler - starling”, one “Schwegler - treecreeper” and one “Schwegler - bat” boxes (for map see Appendix 1). In this experiment, all occupied nest boxes at the KLIVV and the FIWI were included.

2.2.2. Study design

The experiment on the use of odour-manipulated nest material by great and blue tits was conducted during the nest building period until both species started incubation (23rd of March until 27th of April 2015). In the proximity of the 19 blue tit nest boxes at the FIWI and the KLIVV, trials for the odour dependent choice of nest material were installed. One trial consisted of three plastic mesh dispensers each filled with 8 g of either red, green or blue wool charged with odour of lavender (odour group) and three plastic mesh dispensers each filled with 8 g of either red, green or blue wool charged with water (control group). The trials were installed in approximately 3 - 5 m distance to the blue tit nest boxes (Figure 1) (see also Sperandio 2012). The coloured wool of the lavender group was repeatedly treated with two drops of the essential oil (0.2 ml, Primavera life GmbH) and one drop of the hydrolat (0.1 ml) of lavender (*Lavendula angustifolia*) in a 4-day-rhythm. The utilisation of the essential oil and

the hydrolat facilitates standardised dosage and odour intensity is known to be long-term persistent (see also Sperandio, 2012; Weiß, 2013).

The colours of the experimental wool from FILZFAKTOR! (“Bergschafwolle”; coloured according to Ökotextstandard 100: “Scharlachrot”, “Royalblau”, “Grün”) were measured (six measurements per colour) using a USB-2000 spectrometer and a DHS-2000-FHS deuterium halogen lamp which were connected through a bifurcated fibre-optic probe (Ocean Optics, Eerbeek, The Netherlands). Before each measurement the spectro-photometer was calibrated using a standard white (Avantes, Eerbeek, The Netherlands), and for calibration of black the probe was removed from the light source (Mahr et al. 2012).

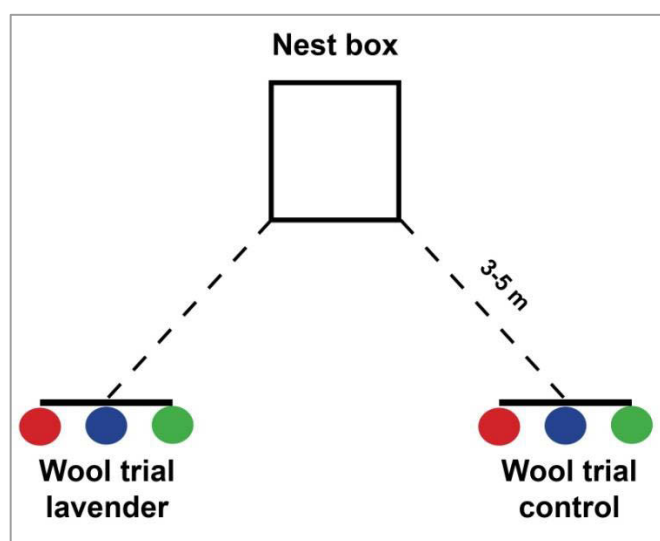


Figure 1: Set-up of the experiment on nest material choice. Wool trials were installed in 3-5 m distance to nest boxes of the type “blue tit” at the FIWI and the KLIVV.

During the experiment, all nests in the study area were visually and olfactory discriminated in a 7-day-rhythm which corresponded to the standard nest controls to reduce disturbance intensity. At the start of the incubation period the trials were removed. The remaining amount of wool in the mesh dispensers were measured using a fine balance (type TP-200, Dipse, max. 200 g, accuracy level 0.01 g) and compared with the 8 g of the initial filling amount to assess the withdrawal quantities.

After the end of the breeding season 2015, the nests were collected and dried in a vacuum drying oven (type UF 260, Memmert GmbH & Co.KG, Schwabach, Germany) at 40°C for 18 hours (Schöll and Hille 2014). The total nests were weighted and manually dissected. The nest compounds were categorised as red wool, blue wool, green wool, other wool, animal origin hair, feathers, moss, twigs, grass, leaves and pine needles. The coloured experimental wool was weighted using a fine balance (type TP-200, Dipse, max. 200 g, accuracy level 0.01 g).

2.2.3. Statistical analysis

Since the data of the experiment were not normally distributed, nonparametric tests were used to proof for differences (Crawley 2013). To test for differences in the withdrawal quantities of the odour-manipulated nest material between the lavender and the control group, the Mann-Whitney U-test was applied. In addition, the Mann-Whitney U-test was used to verify differences in the withdrawal quantities per odour group between the study areas.

To test for differences in the withdrawal quantities and the amount of coloured wool in the nests after the nest dissection, the Kruskal-Wallis-test and the Dunn's test as a post-hoc test were applied (Dinno 2016). The withdrawal quantities per colour and the amount of coloured wool which were retrieved from the nests were tested separately. Additionally, differences of the withdrawal quantities and the amount of retrieved wool between the two study areas were analysed using the Mann-Whitney U-test.

To illustrate the spectrum of the three colours of the experimental wool, the mean value of six measurements per wool colour analysed by the USB-2000 spectrometer (Ocean Optics, Eerbeek, The Netherlands) were used and a spectral curve for each colour was drawn using the R-package "pavo" (Maia et al. 2016). Thus, the colour could be graphically analysed for any reflectance within the ultra-violet range of the light.

The statistical analyses were performed using the software R (3.1.2) (R core team 2014). The significance level for all tests was $p = 0.05$.

2.3. Experiment B - Effects of odour on the breeding performance

2.3.1. Study area

The study areas were situated in the north - eastern part of the Wienerwald in Vienna, Austria, the Kolbeterberg (48.22377° N, 16.24006° E) (Appendix 2) and the Buchberg (48.214167° N, 15.945556° E) (Appendix 3). Deciduous forests, mainly dominated by European hornbeams (*Carpinus betulus*), oak trees (*Quercus spec.*) and beech trees (*Fagus sylvatica*) grow in these areas.

The Kolbeterberg has an area of about 96 ha and is separated in the three parts “Kolbeterberg”, “Kolbeterberg south” and “Kolbeterberg east”. All three parts have different elevation levels and forest structure. The “Kolbeterberg” is an area with an altitudinal gradient of 120 m and characterised by big trees. The “Kolbeterberg south” has an altitudinal gradient of about 50 m and was thinned out in the years 2014 and 2015. The “Kolbeterberg east” is a denser forest with an altitudinal gradient of about 30 m. Since 1959, nest boxes in different arrangement, density and types have been fixed on tree trunks in all areas of the Kolbeterberg. These boxes were rearranged in 2004 and 2005, and now are monitored by the KLIVV in Vienna. A total of 136 wooden nest boxes of different types are located in the three areas of the Kolbeterberg (as per February 2015): 32 boxes of the type “long-old” (180 x 160 x 480 mm, entrance 35 mm), six of the type “long-new” (180 x 185 x 470 mm, entrance 35 mm), 40 of the type “short-old” (165 x 170 x 245 mm, entrance 32 mm), five of the type “short-new” (180 x 185 x 240 mm, entrance 32 mm), five of the type “Urwald” (150 x 160 x 470 mm, entrance 45 mm) and 47 nest boxes for “blue tit” (120 x 155 x 250 mm, entrance 26 mm) and one “woodpecker-nest box” (for map see Appendix 2).

In 2008, the KLIVV installed nest boxes at the Buchberg which has an area of about 21 ha, altitude difference of about 35 m and a similar vegetation structure as the Kolbeterberg. At the Buchberg, a total of 68 nest boxes were installed (as per February 2015): 25 “long”, 22 “short”, one “Urwald” and 20 “blue tit” boxes (for map see Appendix 3).

Nest boxes of the type “long-new” and “long-old” occupied by great tits and “blue tit” boxes occupied by blue tits were included in the experiment to minimise potential interactions of odour and nest box types on the breeding performance of the birds (Winkler 2009). Thus, maximal 85 nest boxes at the Kolbeterberg and 45 nest boxes at the Buchberg could be included in the experiment.

2.3.2. Study design

The experiment on the effects of lavender odour on the nestling condition and nest predation was conducted during the breeding season 2015 (end of March until end of June). Nest boxes with hatching success were randomly assigned to either the lavender or the control group. Nests assigned to the lavender group were continuously treated with two drops of the essential oil lavender (0.2 ml, Primavera life GmbH) and one drop of the hydrolat (0.1 ml) at every nest control. Nests assigned to the control group were charged with 3 drops of water at every nest control. The experiment started with the first day after hatching of the first nestling to minimise stress during hatching time and ended with day 10 (± 1) after hatching.

The hatching date was calculated 13 days after the date of a full clutch under the assumption that each day one egg was produced (Cramp and Perrins 1993). From the calculated hatching day, nests were checked for hatching each day until the first nestling was hatched. Then, nests were controlled in a 7- to 2-days-rhythm depending on the development stage of the nestlings. Nestlings with an average age of 10 days (± 1) were weighted using a fine balance (Model 1479 Tanita, max. 100 g, accuracy level 0.1 g) and the left tarsus was measured using a sliding calliper (accuracy level 0.1 mm) according to Fiedler and Berthold (1999). The nestlings were individually marked using aluminum rings (Diameter (\varnothing) 2.5 mm for great tits, \varnothing 2.5 mm for blue tits) from the German bird ringing Centre Radolfzell.

Adults were caught and ringed when possible, using a trap which was installed at the entrance hole. Standardised measurements of caught adults were taken according to Fiedler and Berthold (1999): left tarsus using a sliding calliper (accuracy level 0.1 mm), the maximum wing length and the length of 3rd primary using a scale (accuracy level 0.5 mm), the body mass using a fine balance (Model 1479 Tanita, max. 100 g, accuracy level 0.1 g), the age and condition (fat and muscle status) of the birds. The disturbance time due to nest control and ringing activity was kept as short as possible. All nests were controlled seven days after ringing to check for fledging, predation or to count dead nestlings. Consequently, the possible number of fledged birds was determined per nest.

2.3.3. Statistical analysis

Prior to the analysis, the mean body mass of the nestlings per nest at day 10 were log-transformed to follow normal distribution. The data showed homoscedasticity. To test for differences of the mean body mass of the nestlings per nest at day 10 between odour-manipulated and control nests, two-sided t-tests were applied for each species separately. Additionally, in order to be able to compare the data of the present study with the data collected in a previous master thesis (Weiß 2013), the Mann-Whitney U-test was applied using the untransformed data of the mean body mass of the nestlings per nest at day 10 (Crawley 2013).

To investigate the effect of lavender odour on preventing the nests from predation, the four-fields-contingency tests (chi-squared test) were performed (Crawley 2013) and differences in the number of predated nests between odours, species and areas were analysed. Additionally, in order to analyse differences in nest predation caused by Aesculapian snake versus other predator species, the Fisher's exact test was applied as best fitting tool due to the small sample size (Crawley 2013).

The statistical analyses were performed using the software R (3.1.2) (R core team 2014). The significance level for all tests was $p = 0.05$.

3. Results

3.1. Odour dependent choice of nest material

During the breeding season 2015, a total of 20 nest boxes were occupied by great tits and six boxes were occupied by blue tits at the FIWI and the KLIVV. Additionally, one box had a finished nest, possible built by a great tit. Thus, the occupation rate was 36 % at the FIWI and 61 % at the KLIVV.

During the five weeks of the experiment, a total of 9.38 g (mean \pm sd: 0.16 ± 0.41 g) of lavender charged wool and 42.55 g (0.75 ± 1.17 g) of non-manipulated wool was removed from the mesh dispensers (Figure 2). Differences in the withdrawal quantities between the odour groups were significant ($p = 0.002$) based on the Mann-Whitney U-test and no significant differences were detected between the areas ($p = 0.149$).

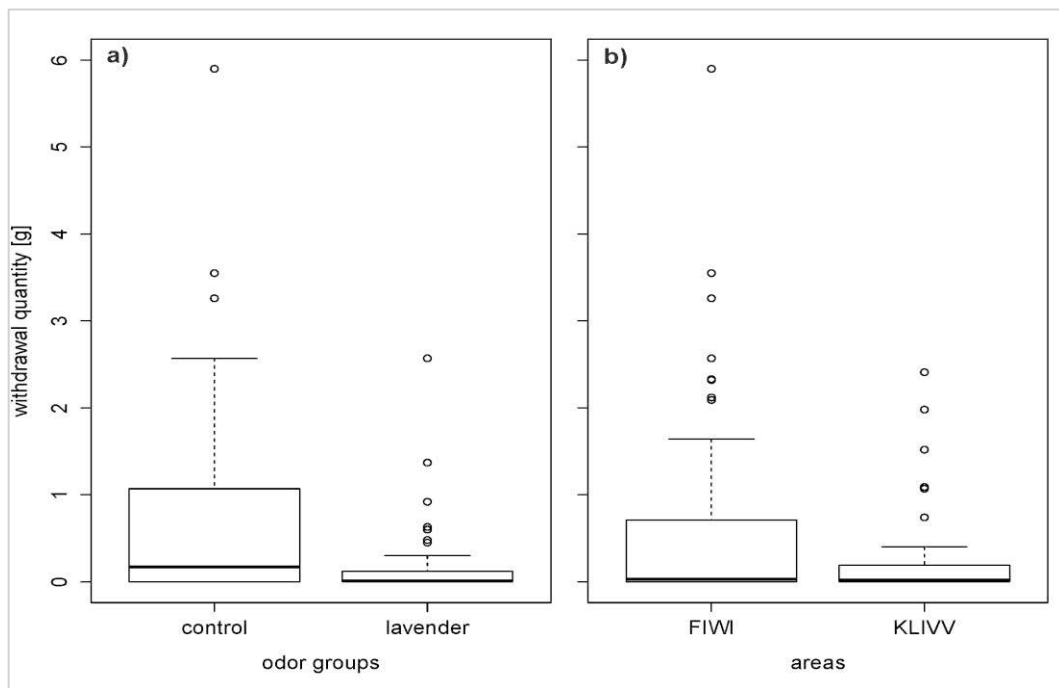


Figure 2: Withdrawal quantities of the offered experimental wool per a) odour groups and b) areas. Sample size was $n = 114$ mesh dispensers filled with wool.

The controls of the nest boxes within the study areas showed that the experimentally offered wool was incorporated in a total of four out of six blue tit nests and 13 out of 20 great tit nests. Additionally, the experimental wool was incorporated in one nest of unknown species. Olfactory discrimination of the nest boxes showed that lavender charged wool was incorporated in five out of 20 nests occupied by great tits and one out of six nests occupied by blue tits as well as in the one built nest of an unknown species.

3.2. Colour dependent choice of nest material

Regarding the colour of the wool, the spectral curve for each colour based on the mean values of the measurements per colours showed high reflectance in its part of the spectrum (red: 600 – 780 nm; green: 490 – 570 nm; blue: 430 – 490 nm) and low reflectance in the ultra-violet spectrum (below 380 nm) (Figure 3).

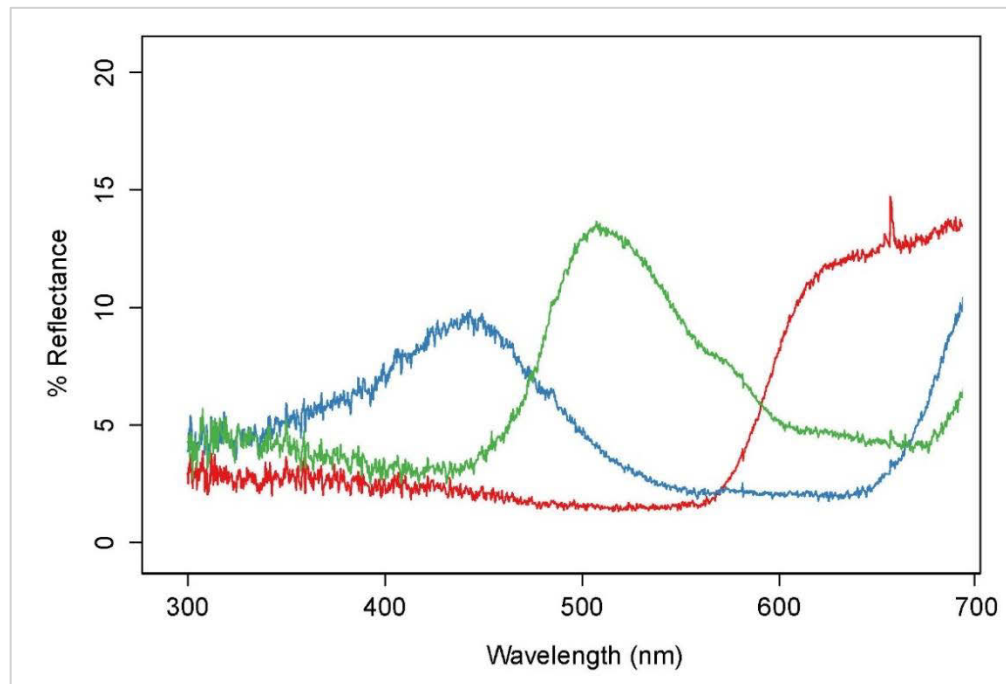


Figure 3: Average spectral reflectance of the three colours of the experimental wool. Lines illustrate the mean values of the colours (six measurements per colour): red line = red colour, blue line = blue colour, green line = green colour. 100 % reflectance is defined as the light reflected from standard white.

In total, 31.81 g (mean \pm sd: 0.83 ± 1.25 g) of red, 8.90 g (0.23 ± 0.53 g) of green and 11.22 g (0.30 ± 0.70 g) of blue wool were removed from the mesh dispensers (Figure 4). Differences in the withdrawal quantities between the three colours were significant based on the Kruskal-Wallis test ($\chi^2 = 13.009$, $df = 2$, $p = 0.001$). The following Dunn's tests showed significant differences in the withdrawal quantities between red and green wool ($p < 0.001$) and between red and blue wool ($p = 0.003$). No significant differences could be detected between green and blue wool ($p = 0.292$).

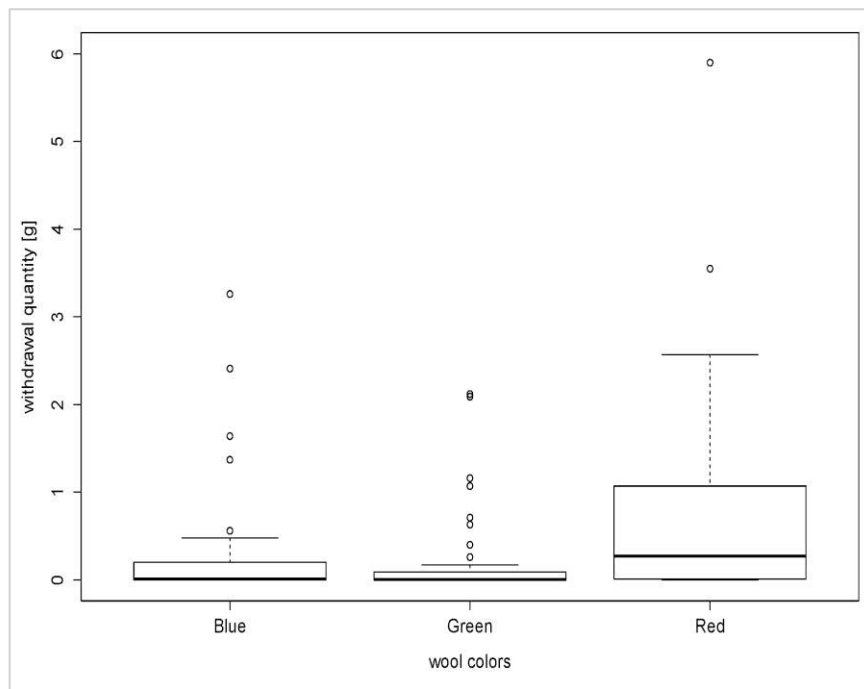


Figure 4: Withdrawal quantities of blue, green wool and red wool. Sample size was n = 114 mesh dispensers filled with wool.

Out of the 27 dissected nests, the experimental wool was incorporated in a total of 18 nests. In total, 14.24 g (0.79 ± 1.14 g) of red wool, 4.83 g (0.27 ± 0.56 g) of green wool and 5.93 g (0.33 ± 0.80 g) of blue wool could be retrieved (Figure 5). Thus, about 48.14 % of the withdrawn wool could be retrieved within the 18 nests.

Differences in the amount of retrieved wool between colours were significant based on the Kruskal-Wallis test ($\chi^2 = 9.315$, $df = 2$, $p = 0.009$). The following Dunn's test showed significant differences in the retrieved wool between red and green wool ($p = 0.004$) and between red and blue wool ($p = 0.005$). No significant differences could be detected between green and blue wool ($p = 0.474$). Differences in the amount of retrieved wool between the areas showed no significant differences ($p = 0.09$) based on the Mann-Whitney U-test.

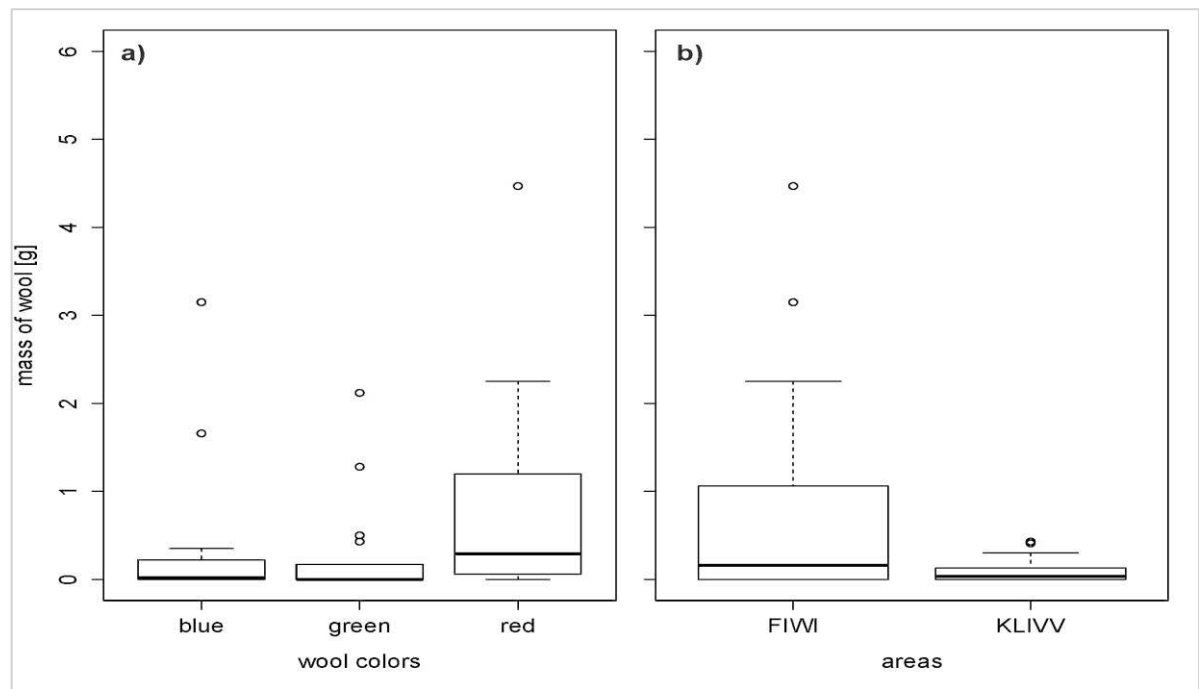


Figure 5: Amount of retrieved coloured experimental wool of the nest boxes per a) colour and b) areas. Sample size was n = 18 nests.

In addition, all 27 occupied nests were analysed for its nest material (Figure 6). All 27 nests consisted of moss. Animal origin hair (from undefined species) was found in 26 nest boxes and feathers and twigs were found in 21 nests. In all 18 nests with retrieved experimental wool, red wool was incorporated. Furthermore, nine nests had blue wool and eight nests had green wool incorporated. In seven nests one colour, in six nests two colours and in five nests all three colours of the experimental wool were incorporated. In a total of 12 nests, non-experimental wool with the colours white, grey, yellow or orange were retrieved.

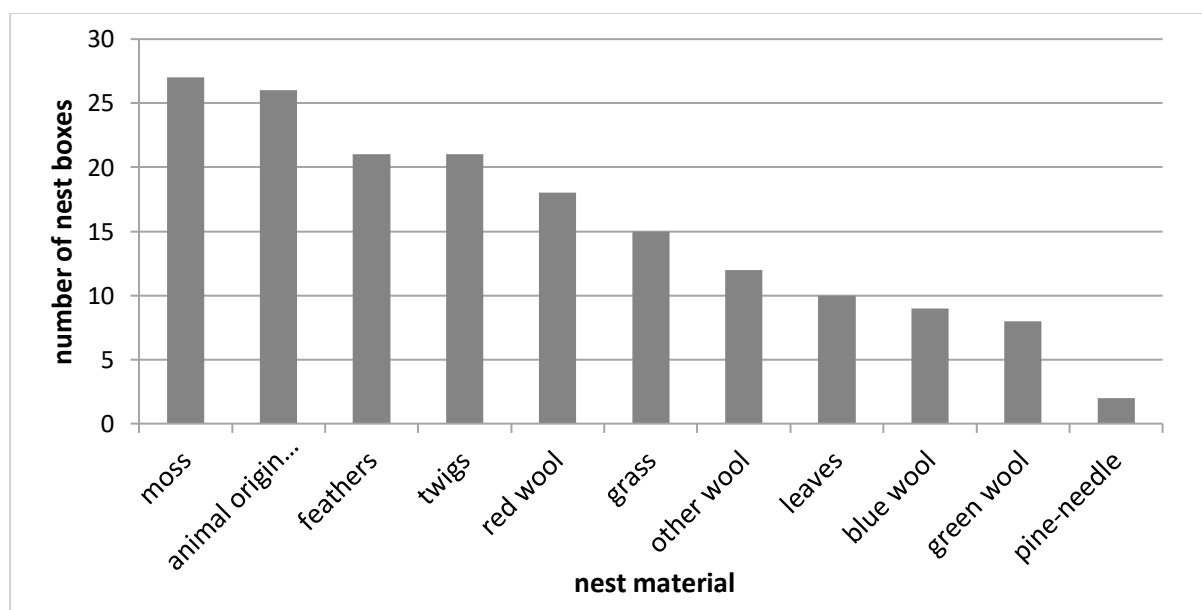


Figure 6: Overview of the results of the nest dissection. Nest compounds found in the number of nest boxes. Sample size was n = 27 nests.

3.3. Effect of odour on nestling condition

At the Kolbeterberg, a total of 28 nest boxes of the type “long” were occupied by great tits and 41 “blue tit” nest boxes were occupied by blue tits. At the Buchberg, a total of 22 nest boxes of the type “long” were occupied by great tits and a total of 17 “blue tit” nest boxes were occupied by blue tits. At Kolbeterberg, 12 nest boxes and at Buchberg, six boxes were predated before hatching and three clutches were abandoned before hatching and therefore not included in the odour experiment. In total, hatching success could be monitored for 55 nest boxes at the Kolbeterberg and 31 nest boxes at the Buchberg. These boxes were randomly assigned to the lavender treated or the control group (Table 1).

Table 1: Overview of the distribution of nest boxes included in the experiment on the effects of lavender odour on the breeding performance. Great tits: “long” nest boxes with hatched great tits; Blue tits: “blue tit” boxes with hatched blue tits. Number of nest boxes per study areas and odour groups.

	Lavender nests	Control nests	Sum nests
Great tits at Kolbeterberg	12	13	25
Blue tits at Kolbeterberg	19	11	30
Great tits at Buchberg	7	8	15
Blue tits at Buchberg	8	8	16
Sum	46	40	86

In total, 46 boxes at the Kolbeterberg and 26 boxes at the Buchberg could be monitored until day 10 of the nestlings. Contrary to study protocol, nestlings at the Buchberg were not ringed and measured. Reason for this protocol deviation were communicational misunderstandings between ringing persons. Five “long” nest boxes occupied by great tits and four blue tit boxes had to be excluded from analyses because the average nestling age was beyond 10 (± 1) days. Therefore, the body mass of nestlings with the average age of 10 (± 1) days were analysed for 18 great tit nest boxes and 19 blue tit nest boxes with a total number of 337 nestlings (Table 2). The number of nestlings ranged from 5 -12 in both great tits (mean \pm sd: 9.3 ± 2.1) and in blue tits (8.9 ± 2.5).

Table 2: Overview of the distribution of nest boxes and number of nestlings included in the analyses of the influence of lavender on the nestling condition at the Kolbeterberg. Sample sizes were n = 18 nests for great tits and n = 19 for blue tits.

	Lavender nests	Control nests	Sum nests	Lavender nestlings	Control nestlings	Sum nestlings
Great tits at Kolbeterberg	9	9	18	74	93	167
Blue tits at Kolbeterberg	12	7	19	105	65	170
Sum	21	16	27	179	158	337

The overall mean body mass of nestlings was 15.68 g (\pm sd: 1.13 g) for great tits and 9.92 g (\pm 1.21 g) for the blue tits. The body mass of great tit nestlings of the lavender treated group (mean body mass \pm sd: 16.15 \pm 1.18 g) was on average 0.95 g higher compared to control group nestlings (mean body mass \pm sd: 15.20 \pm 0.90 g) (Figure 7, a). The body mass of blue tit nestlings of the lavender treated group (mean body mass \pm sd: 9.96 \pm 1.24 g) was on average 0.11 g higher compared to the control group nestlings (mean body mass \pm sd: 9.86 \pm 1.24 g) (Figure 7, b). The two-sided t-test using mean of the log-transformed body mass of the great tits (Figure 7, c) and of the blue tits (Figure 7, d) showed neither significance differences between the odour groups for the great tits ($t = -1.871$, $df = 15.11$, $p = 0.080$) nor for the blue tits ($t = 0.526$, $df = 10.84$, $p = 0.610$). Using the untransformed mean body mass at day 10, the Mann-Whitney U-test showed significant differences for the great tits ($p = 0.050$) and no significant differences for the blue tits ($p = 0.967$) between the odour groups.

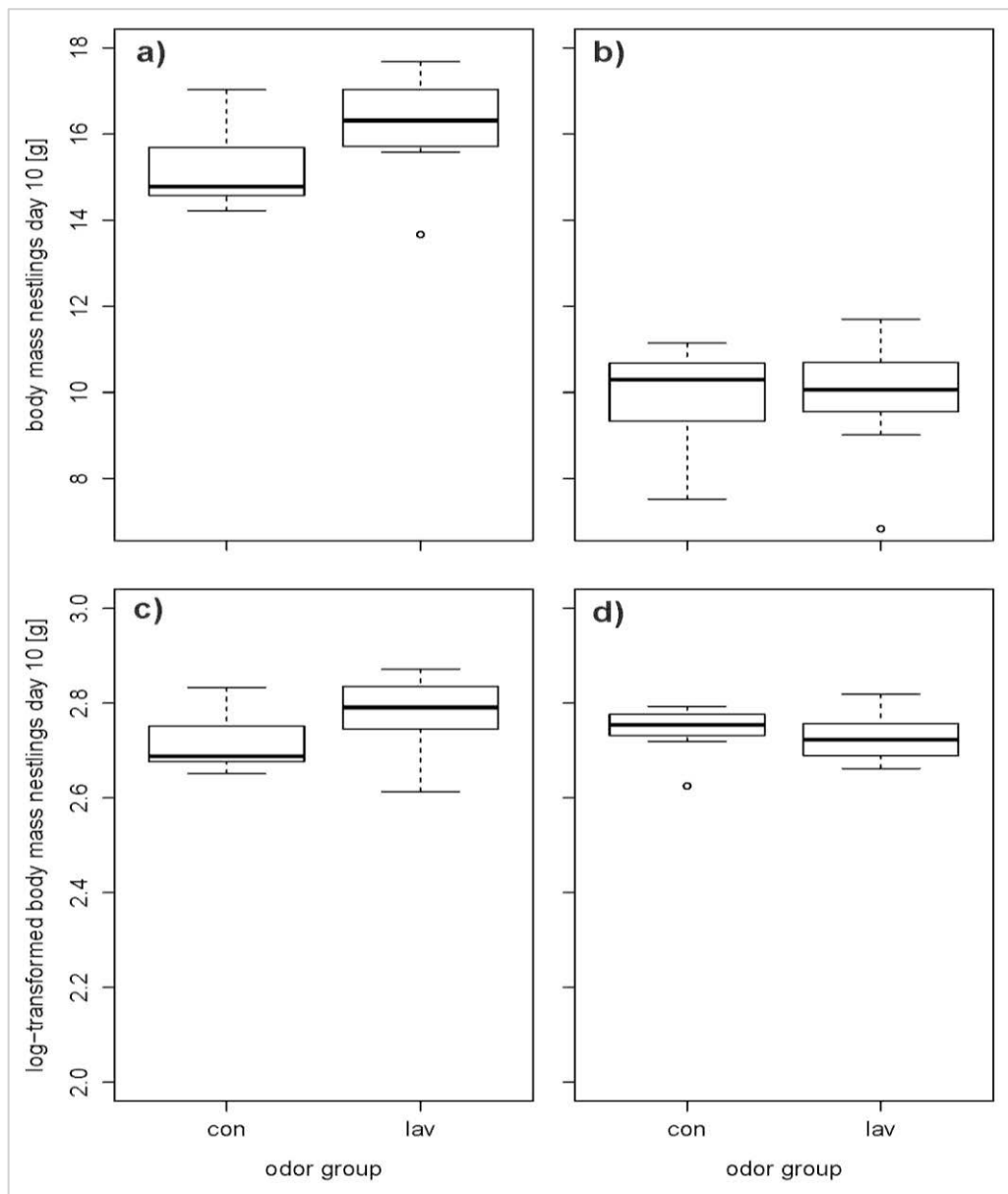


Figure 7: Mean body mass of nestlings at day 10 (untransformed) of a) great tits and b) blue tits per nest box at the Kolbeterberg. Mean body mass of nestlings at day 10 (log-transformed) of c) great tits and d) blue tits per nest box at the Kolbeterberg. Con = control group, lav = lavender treated group. Sample sizes were $n = 18$ nests for great tits and $n = 19$ for blue tits.

3.4. Effect of odour on nest predation

In total, 55 nest boxes at the Kolbeterberg and 31 nest boxes at the Buchberg were included in the nest odour-manipulation experiment. These boxes were randomly assigned to the lavender treated or the control group (Table 1). During the experiment, a total of 14 boxes at the Kolbeterberg and 11 boxes at the Buchberg were predated. The majority of predated nest boxes belonged to the control group whereas most often nestlings of boxes charged with lavender odour survived and potentially fledged (Table 3). The results of the Chi-squared test (Table 3) showed significant differences between the odour group ($p = 0.001$) and missed the level of significance regarding the areas ($p = 0.462$) and the species ($p = 0.591$).

Table 3: Differences in nest predation between areas, species and odour groups. Significances were calculated based on Chi-squared test (with Yate's continuity correction). Sample size was $n = 86$ nests.

		Predated	Survived	Chi ²	df	p-value
Area	Buchberg	11	20	0.542	1	0.462
	Kolbeterberg	14	41			
Species	Great tit	10	30	0.288	1	0.591
	Blue tit	15	31			
Odour	Lavender	6	40	10.705	1	0.001
	Control	19	21			

Regarding the six predated, lavender treated nests (Table 3), one was predated by the Aesculapian snake, one by a squirrel, two by woodpecker and two by unknown predators. Out of the 19 predated control nest boxes, one nest each was predated by a woodpecker, by a squirrel and by an unknown predator. The 16 remaining predated nests of the control group were predated by Aesculapian snakes. When testing the predation of snake versus the other predators regarding the odour groups, the Fisher's exact test showed significant results ($p = 0.006$) (Table 4).

Table 4: Differences in predation between odour groups regarding predation by Aesculapian snakes versus other predators. Significances were calculated based on Fisher's Exact test. Sample size was $n = 25$ nests.

		Aesculapian snakes	Other species	Sum	p-value
Odour	Lavender	1	5	6	0.0055
	Control	16	3	19	

4. Discussion

In this study, I conducted two independent experiments to demonstrate whether the choice of nest material was odour and/or colour dependent and whether the specific odour of lavender affected the nestling condition and the nest predation rate of free-ranging great and blue tits in Vienna. My results showed that both odour and colour affected the choice of nest material of great and blue tits, and that lavender had a positive effect on the potential breeding success of both species.

4.1. Odour dependent choice of nest material

The experiment on the odour dependent choice of nest material showed that non-manipulated material was significantly chosen more often based on the withdrawal quantities of the experimental wool. Furthermore, lavender charged wool could only be detected in seven out of 27 occupied nests and 9.38 g of odour charged wool were withdrawn in total. These results indicate that tits were able to discriminate between the lavender and non-manipulated material, but preferentially choose the non-manipulated nest material. This demonstrates for the first time that olfaction affects great and blue tits in Central Europe when collecting nest material. Until now, this effect was only shown for European starlings (Clark and Manson 1985; Clark and Mason 1987; Clark 1990; Gwinner et al. 2000) and Corsican blue tits (Lambrechts and Dos Santos 2000; Petit et al. 2002; Mennerat et al. 2005; Mennerat 2008; Pires et al. 2012).

During the breeding season 2015, most of the nest boxes in the study areas were occupied by great tits (20 out of 27 occupied nest boxes). Based on the significant results of the choice of the non-manipulated nest material and the low amount of great tit nests with lavender charged wool, the results indicate that great tits are also able to recognise the odour of lavender. It is questionable why the breeding tits avoided the lavender charged nest material because in a previous master's thesis, no odour dependent choice of the offered nest material was reported (Sperandio 2012). This discrepancy may be due to differences in the study design. Contrary to Sperandio (2012), in the present study one drop of lavender hydrolat was additionally used to odour-manipulated nest material. Therefore, the nest material in this study had a more intense odour than in the study by Sperandio (2012), which might have an influence on the ability of great and blue tits to detect the odour of lavender. Further, Sperandio (2012) offered dog's hair in the experiment whereas I used coloured wool. Therefore, the structure of offered nest material differed between the two studies. The choice

of odour-manipulated coloured wool was shown to be affected by the odour whereas the choice of odour-manipulated dog's hair was not (Sperandio 2012).

Another explanation is that the great and blue tits in my study area are not familiar with the odour of lavender because it is not as frequently available in Central Europe like it is in Mediterranean regions (e.g. Corsica). Therefore, the birds in my study area might avoid the lavender charged nest material because of some effect of neophobia which is not likely to occur in case of the Corsican blue tit based on the availability of lavender in the study areas of those studies (Lambrechts and Dos Santos 2000 ; Petit et al. 2002; Mennerat et al. 2005; Mennerat 2008; Pires et al. 2012). Neophobia, or the fear of novel objects, is a common behaviour and often found in wild animals (Fischer et al. 2016; Turini et al. 2016), but most studies focus on the role of neophobia in foraging behaviour (e.g. Marples et al. 1998; Fischer et al. 2016). A study focusing on neophobic behaviour towards novel food (colour and/or odour manipulated) showed longer latency of approaching the food in situations of both colour-and-odour-manipulated food, but no effect of only odour-manipulated food in zebra finches (Kelly and Marples 2004a). Regarding the possible neophobic behaviour towards lavender, Sperandio (2012) recorded longer latency behaviour of entering the nest boxes in the first visits which decreases over successful approaches in both males and females.

However, the assessment of neophobia requests detailed observations of the behaviour because at the point of the first approach to the novel object, neophobia has already been overcome (e.g. Kelly and Marples 2004a). Thus, an effect of neophobia towards lavender charged nest material remained hypothetical in my study. Nevertheless, the avoidance of the odour of lavender was interesting because blue and great tits are reported to use a broad assortment of nest materials, especially for the nest-lining (e.g. Hansell 2000; Surgey et al. 2012; Schöll and Hille 2014) and therefore are assumed to have a lower level of neophobia than other species (e.g. Fischer et al. 2016).

4.2. Colour dependent choice of nest material

Regarding the colour of the wool, red wool was withdrawn in a significant higher amount than blue and green wool. However, based on the withdrawal quantities of the coloured wool, it is not possible to say which taxa withdrew the wool because besides blue and great tits, other bird species are present in the study area as well as mammals (e.g. squirrels, mice) which might also use the coloured wool for diverse reasons. Therefore, the amount of coloured wool in the nests is a better indication for the choice of nest material in blue and great tits. In the

18 nests with coloured wool, I found about 50 % of the withdrawn wool with also the highest amount of red wool, followed by blue and green wool.

According to my hypothesis, the colours had a significant influence on the choice of nest material. A possible effect due to any ultra-violet reflectance of the material could not be demonstrated because none of the three colours showed any peak in the reflectance below 380 nm. To my knowledge, a colour-dependent choice of nest material has not yet been reported for blue and great tits. Surgey et al. (2012) only showed a tendency that great and blue tits might prefer red coloured wool over green, white and yellow wool.

My results agreed with this tendency and showed that red wool was chosen significantly more often than blue and green wool. Interestingly, red wool was found in 18 nest boxes, thus red wool was amongst moss, animal origin hairs, feathers and twigs a favoured nest material for blue and great tits in my study. The higher choice of red wool might be explained by the higher contrast of red with the background of forests and shrubs (Camargo et al. 2013; Schmidt et al. 2004), and therefore, the red material might be more attractive for birds (Marples et al. 1998) and is more easily recognised visually at distance (Gamberale-Stille et al. 2007). The colour red is a common colour signal and is reported to be an important colour in diverse biological contexts of the life of passerines. On one hand, the colour red is a so-called “warning colouration” or “aposematic colouration” when the bird associated unpalatable or toxic prey with the colour red (Marple 1998). For instance, wild-caught great tits were demonstrated to avoid red-and-black insects which might be based on previous individual learning in the wild (Hotova Savadova et al. 2010). On the other hand, red is besides black an attractive fruit colour because it is conspicuous against the natural background (Gamberale-Stille et al. 2007; Camargo et al. 2013; Schmidt et al. 2004). Based on the study by Schmidt et al. (2004), the conspicuousness and not the colour influences the choice of a coloured object (“contrast hypothesis”). In a choice experiment, birds preferred food which contrasts with the background (red with green background and *vice versa*) (Schmidt et al. 2004). Considering the “contrast hypothesis”, red coloured wool might be chosen over green and blue wool because of the higher contrast to the background as demonstrated for the choice of fruits in birds (Schmidt et al. 2004; Camargo et al. 2013). Furthermore, the lack of significant differences between green and blue coloured wool supports the notion of Muth et al. (2013) that birds might have difficulties to differentiate between green and blue because both colours are close to each other in the spectral spectrum.

Nevertheless, great and blue tits build their nest of green material (moss; e.g. Cramp and Perrins 1993) and European starlings and Corsican blue tits are reported to incorporate aromatic herbs in their nests (e.g. Clark and Manson 1985; Lambrechts and Dos Santos 2000). An explanation for the low amount of green coloured wool chosen in my experiment is that birds might use green material due to the structural function of it (e.g. important influence on insulation; Schöll 2013) rather than the colour of material (Muth et al. 2013; Muth and Healy 2014, 2011). Regarding the colour, an effect of neophobia due to the colours green and blue are not likely to occur. In 2014, various colours of wool, including white, yellow, green, blue, orange, pink, red, and violet were incorporated in the nests at the Kolbeterberg (*personal observation 2014*).

5.3. Effect of odour on nestling condition

The experiment on the effects of lavender on the nestling condition showed that great tits at day 10 were on average about 1 g heavier in lavender-manipulated nests compared with non-manipulated ones. The result missed level of significance, though a tendency of the effect of lavender could be demonstrated. Regarding blue tits, the result showed also no significant effect of lavender on the body mass of the nestlings at day 10. Blue tit nestlings were on average 0.11 g heavier in lavender charged nests. These results are possibly influenced by the low sampling size with 18 great tit nests and 19 blue tit nests at the Kolbeterberg.

Interestingly, a previous master's thesis by Weiß (2013) showed significant differences between the body mass of blue tit nestlings, although the sampling size was also low with 34 nest boxes at the Kolbeterberg and 18 nest boxes at the Buchberg. These different results could be explained by i) differences in parasite pressure due to seasonal differences between years (Mennerat et al. 2009a) and ii) the use of untransformed data of the body mass and consequently the use of a non-parametric test (Mann-Whitney U-test) due to not normally distributed data. When I also used the untransformed data of the body mass of the nestlings and performed the Mann-Whitney U-test, the tendency of higher body mass of great tits in lavender-manipulated nests reached the level of significance ($p=0.050$). The results of the body mass of the blue tit nestlings remain non-significant in my study. Hence, my study could not draw line with the results of Weiß (2013), even when using the same statistical analysis. Thus, differences between the studied years are more likely to cause the different results.

In addition, differences between areas are likely to occur regarding the parasite pressure of nests and nestlings due to differences in the nest composition between areas

which affect parasite abundance (Mennerat et al. 2009b; Moreno et al. 2009; Cantarero et al. 2013). In 2015, the nests at the Kolbeterberg had no obvious unusual parasite loads, except for some blue tit nests where many mites were present and the nestlings died probably due to the parasite pressure. At the Buchberg, the parasite pressure was possibly higher as observed during nest controls. However, these observed differences remain hypothetical because no standardised measurement of parasite pressure was conducted during the breeding season 2015.

My results draw level with many studies that also failed to show a significant effect of aromatic herbs on the body mass of the nestlings (Wimberger 1984; Shutler and Campbell 2007; Mennerat et al. 2009b; Tomás et al. 2012). However, the lack of a significant effect on the body mass of the nestlings does not necessarily mean that the odour of lavender had no effect on the nestling condition or reduction of the parasites in the nest. Previous studies showed that although the body mass did not increase, the aromatic herbs within the nest had positive effects on the immune system (Gwinner et al. 2000) or reduced the parasites loads (Shutler and Campbell 2007; Tomás et al. 2012). However, these parameters were not included in my study, therefore a relationship between the odour of lavender and, e.g. the haematocrit levels or the number of parasites remains hypothetical. Therefore, the “nest protection hypothesis” and the “nest drug hypothesis” could neither be totally supported nor rejected for great tits and blue tits in my study area based on the available data.

5.4. Effect of odour on nest predation

The experiment on the effect of lavender on the nest predation showed significant results. Nest boxes charged with lavender odour were significantly fewer predated than nest boxes belonging to the control group. This result agreed with my hypothesis that lavender has a positive effect on the breeding success by lowering the nest predation rate. Furthermore, the test focusing on the predator species showed also significant results. Out of the six predated nest boxes of the lavender group, only one was predated by Aesculapian snake. The other 16 nest boxes predated by Aesculapian snake belonged to the control group. Therefore, my results showed a first indication that lavender is a prevention from nest predation by Aesculapian snake whereas other predators (e.g. woodpecker, squirrel) seemed to be unaffected by the odour. This result agrees with a previous study that lavender odour functioned as a snake repellent, although the study by Clark and Shivik (2002) focused on the brown treesnake. Additionally, previous master theses on the effect of lavender as predator

repellent showed a tendency that the predation by Aesculapian snake is affected by lavender, though previous results missed level of significance (Sperandio 2012; Weiß 2013). To my knowledge, my study is the first one to show significant repellent effects of lavender odour on the Aesculapian snake.

Snakes are known to have low visual and auditory (mainly vibration) abilities based on their anatomy, and strike only on moving prey (e.g. Gruber 1989). Thus, the sense of smell is the most important sense in snakes and most often the major sense in finding their prey (e.g. Gruber 1989). The major olfactory organ of snakes is the tongue which directs odorous compounds to the so-called “Jacobson’s organ” which is located in the palatine and processes the odour input (Gruber 1989; Mattison 1999). Aesculapian snakes are active foragers and searches for their prey in, e.g. tree-holes, and constrict their prey (Mattison 1999). During the breeding season of birds, Aesculapian snakes switch their diet from small mammals to nestlings and eggs (Glandt 2010). In my study area, the Aesculapian snake is, beside mammals and woodpeckers, a major predator of birds breeding in nest boxes. Based on the foraging behaviour and the used senses of snakes in finding their prey, it is likely that the odour of lavender masks the odour cues used by Aesculapian snakes to find the nestlings, alike it is proposed for ectoparasites to find their hosts (Wimberger 1984). Additionally, lavender is reported to cause aggressive behaviour when exposed to the odour (Clark and Shivik 2002). Therefore, snakes might avoid the exposure to the odour.

Nevertheless, studies focusing on the forage behaviour of Aesculapian snakes in context with the effect of lavender are needed to fully understand the potential role of the odour in predation avoidance. As nest predation is a primary source of the reproductive failure for passerines (Cox et al. 2013; Sperry et al. 2009; Klug et al. 2010; Quan and Li 2015), the role of lavender as an Aesculapian snake repellent might be of interest in areas with a high density of snakes in conservation management of, e.g. declining or endangered bird species.

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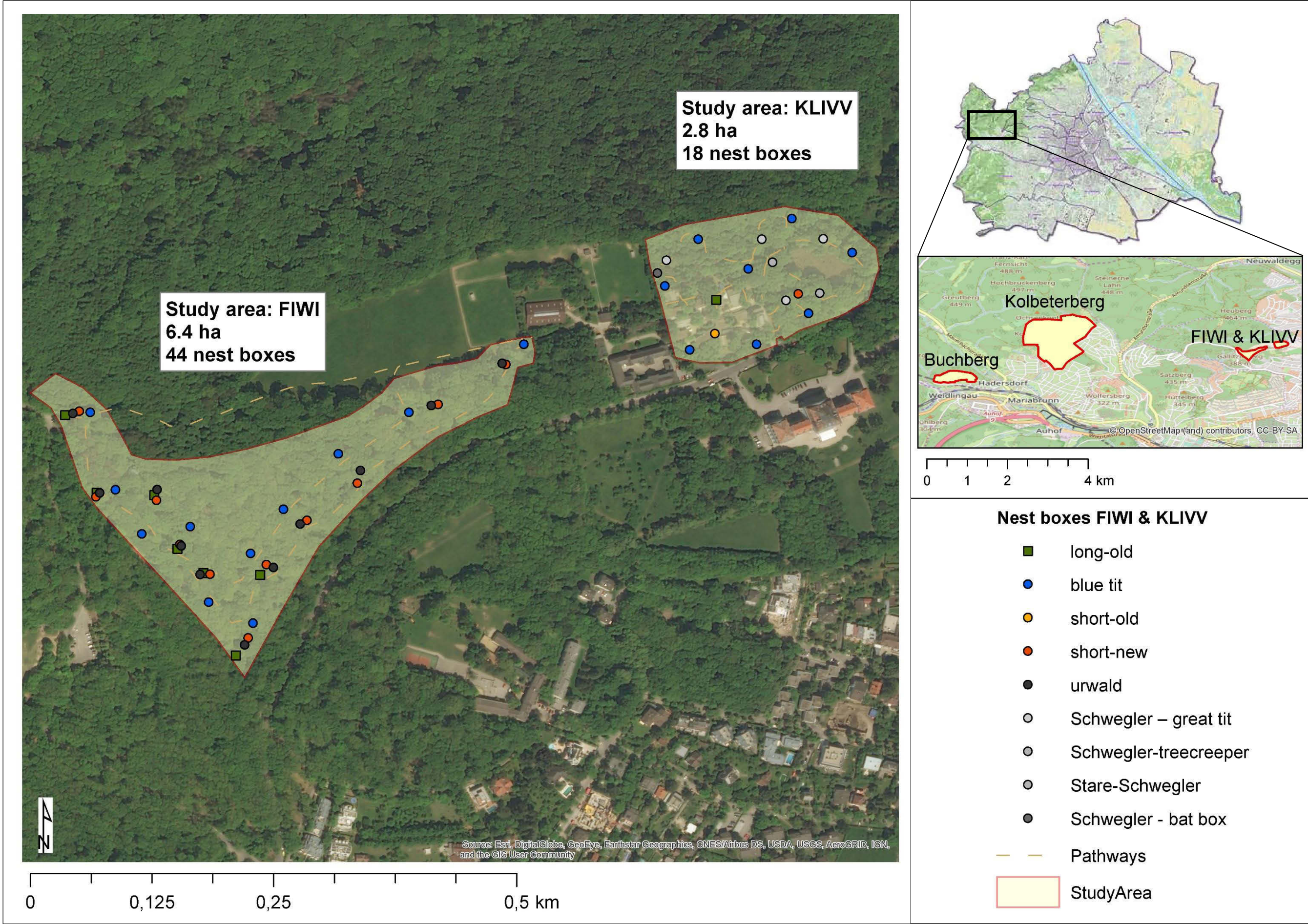
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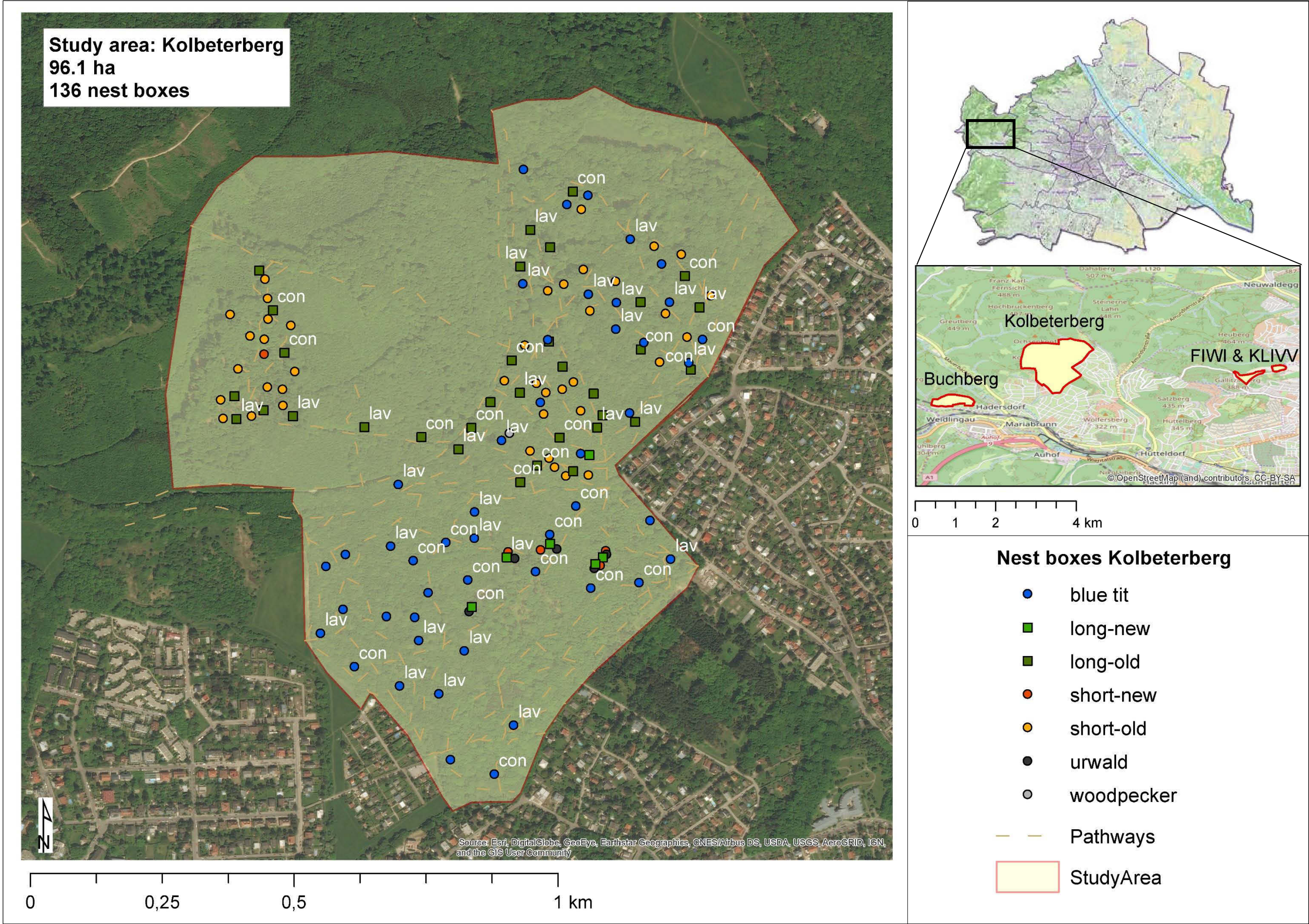
7. Appendices

Appendix 1: Overview of the distribution of the nest boxes at the FIWI and the KLIVV	40
Appendix 2: Overview of the distribution of the nest boxes at the Kolbeterberg.....	41
Appendix 3: Overview of the distribution of the nest boxes at the Buchberg	42

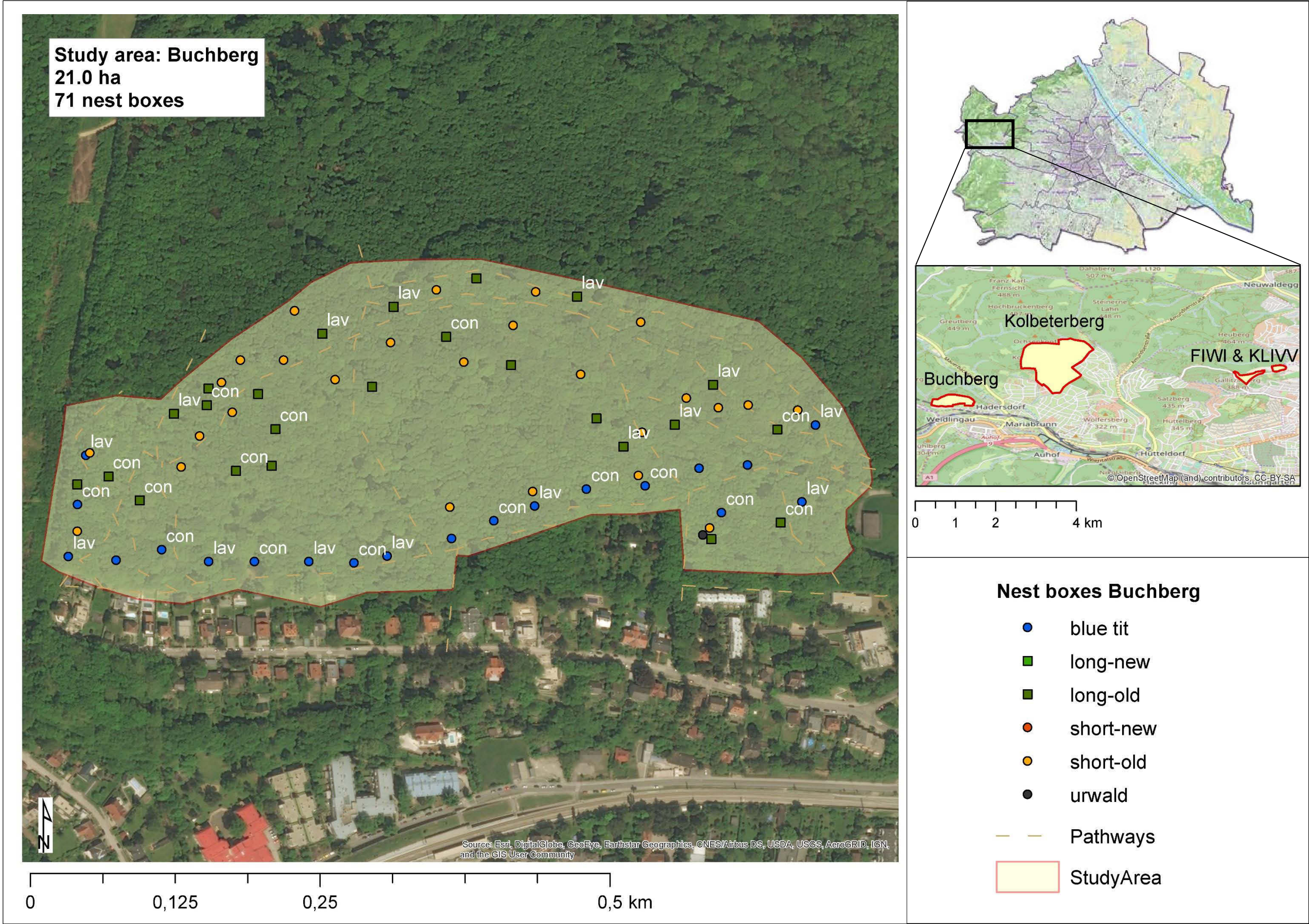
Appendix 1: Overview of the distribution of the nest boxes ate the FIWI and the KLIVV (left) and the location of study areas in Vienna, Austria (right). Created using ArcMap Version 10.2. (Esri 2011).



Appendix 2: Overview of the distribution of the nest boxes at the Kolbeterberg (left) and the location of study areas in Vienna, Austria (right). Created using ArcMap Version 10.2. (Esri 2011).



Appendix 3: Overview of the distribution of the nest boxes at the Buchberg (left) and the location of study areas in Vienna, Austria (right). Created using ArcMap Version 10.2. (Esri 2011).





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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.

03-Feb-2017

Date

Signature