The first wolf found in the Netherlands in 150 years was the victim of a wildlife crime

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Abstract: On July 4th 2013 a dead subadult female wolf-like canid was found by the roadside between Luttelgeest and Marknesse in the Noordoostpolder in the central part of the Netherlands. As the last observations of wild wolves in the Netherlands date back to 1869 the discovery of this animal generated a lot of media attention. European wolf populations have been expanding since the 1950s and the first packs recently established themselves in Germany in geographic proximity of the Dutch border, so natural re-appearance of the species in the Netherlands seemed likely. We investigated the taxonomy of the animal, its geographical origin, and its most recent history. Macroscopic and biochemical analyses of the dead animal convincingly showed that it was a purebred wolf, related to populations from eastern Europe. Bullet impacts and shattered fragments found in the chest and flank, and a discrepancy between the timing of the *post mortem* and *rigor mortis* intervals indicated that this wolf was shot prior to illegal transport to the Netherlands. The wolf fed on beaver in either the Carpathian mountains or the Eifel which is too far for the animal to have walked from by itself within the 24 hours needed to digest its last meal. These geographical areas are the only regions where haplotypes and $^{87}Sr/^{86}Sr$ isotopes retrieved from both the dead wolf and the beaver remains in its stomach co-occur. We therefore conclude that the first Dutch wolf found in the Netherlands in 150 years did not enter the Netherlands by itself but sadly proved to be the victim of wildlife crime.

Keywords: Canis lupus, Europe, haplotypes, isotopes, microsatellites, wildlife forensics, wolf.

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Introduction

On July 4th 2013, a large dead canid was found along the Uiterdijkenweg between Luttelgeest and Marknesse (RD-coordinates 191.1 - 528.6) in the Noordoostpolder in the central part of the Netherlands (figure 1). Although the wolf (*Canis lupus*) was once widespread throughout Europe, its natural range was reduced to approximately one third of its original size during the last centuries due to human persecution. Recovery of European wolf populations began during the 1950s when traditional rural economies declined and the need to kill wolves decreased (Boitani 2000). Currently, several main metapopulations in Europe exist from which wolves are colonising new territories from different directions (Stronen et al. 2013). The first metapopulation is formed by the approximately 2000 wolves inhabiting the Iberian Peninsula, of which 150 live in northeastern Portugal (Bessa-Gomes & Petrucci-Fonseca 2003). The second metapopulation originated from an expansion of the Italian Alpine population. That led to recolonisation of the western Italian and French Alps, recently followed by several new regions (Pyrenees, Jura, Vosges, Massif Central and Catalonia). This metapopulation now comprises about 800 animals (Caniglia et al. 2012, Fabbri et al. 2014). The third metapopulation consists of wolves from central Sweden that have expanded into southern Norway and contains at least 200 animals. The fourth metapopulation is formed by northcentral European wolf populations in Germany, Estonia, Latvia, Lithuania, Finland and Russia and currently encompasses over 3500 animals (Baltrunaite et al. 2013, Czarnomska et al. 2013). The fifth metapopulation is situated in the Ukraine. The sixth metapopulation is formed by wolves living in the Carpathian mountains that are situated in the Czech Republic, Poland, Romania, Slovakia and Ukraine. The seventh metapopulation is situated in the Balkans (Albania, Bosnia-Herzegovina, Bulgaria, Greece, Macedonia, Montenegro and Serbia). The numbers of animals of the last three metapopulations are not known.

Multiple fossil remains show that wolves lived throughout the Netherlands up to the

Figure 1. Dead wolf found by the road between Luttelgeest and Marknesse (Noordoostpolder) in the Netherlands on July 4th 2013. *Photo: Janneke van der Linde.*

Figure 2. Remains of dead wolf prior to section at DWHC and now deposited in Naturalis Biodiversity Center (RMNH.MAM.5000081). *Photo: Hugh Jansman.*

Pleistocene (http://www.geologievannederland.nl/fossielen/zoogdieren/wolf). Wolves are regularly mentioned in various historic documents, although a gradual decline set in once agriculture became more widespread. In some cases there were short-lasting invasions of wolves, as in the winter of 1233-1234 in the east of the Netherlands where buried bodies had to be protected to prevent them from being scavenged (Picardt 1660). In periods with low human population density wolves increased their distribution in the Netherlands, for example after the Black Death, and during the Eighty Years' War (1568-1648) (de Rijk 1985). In 1869, two wolves were caught in Schinveld (Limburg) in the south of the Netherlands (Flaton 1989). According to Okarma (2000), the last wild wolf of that century was observed nearby in Heeze (Brabant) in 1897. However, this record is disputed because by that time wolves had already been exterminated in the Ardennes and the Eifel.

From the beginning of the information campaign 'Wolves in the Netherlands' (http://www.wolveninnederland.nl) in 2008, claims of wolves and wolf tracks were reported on a regular basis. On and around 27 August 2011 actual sightings (and photographs) of an animal similar in morphology to a wild wolf were reported near Duiven in the east of the Netherlands. Following the media attention on wolves when a claim is reported, the number of wolf sightings or tracks increases, to decrease again to the normal level of approximately one claim per week in the months afterwards. Between 2011 and 2013, wolf sightings have also been reported in Belgium and Denmark, some documented by camera traps, all presumed to be the result of natural migrations from adjacent countries. In 2011 a wolf was filmed near Namur, in the Belgian Ardennes. In December 2012, a dead wolf was found in Denmark (Madsen et al. 2013). DNA analysis proved that this animal was born in Germany and had migrated at least 850 km (C. Nowak, unpublished data). In 2011, a wild wolf was photographed in the Westerwald in Germany, just 130 km from the Dutch border. This animal was accidentally shot in April 2012. DNA analysis showed that this animal was derived from the Alpine wolf populations. In April 2013, a wild wolf was photographed with a camera trap near Meppen in Germany, just 30 km from the Dutch border.

In 2012 a minimum of 14 wolf packs was living in Germany and in August 2013 a total of 23 packs was recorded. The nearest wolf pack is situated at approximately 150 km from the Dutch border and juvenile wolves commonly disperse over long distances (100-150 km, with some individuals dispersing up to 1500 km (Ciucci et al. 2009)). The increasing number of sightings along the Dutch-German border is therefore not surprising.

Detailed investigations on the remains of the dead canid found in the Noordoostpolder were carried out to come up with (1) a sound taxonomic identification, (2) reconstruction of its geographical origin, and (3) its most recent history to assess whether this was the first wolf re-appearing in the Netherlands after 150 years or a victim of a wildlife crime.

Materials & Methods

Macroscopy

Section - After discovery the carcass was transported to Utrecht where it was sectioned (figure 2) and inspected by representatives of Alterra WUR, The Dutch Wildlife Health Centre and Naturalis Biodiversity Center. Blood, hide, skeleton, stomach content and tissue samples were frozen for further research.

Biochemistry

DNA analyses - Wolves and dogs are genetically very similar due to domestication of dogs from wolves, which only started ca. 40,000 years ago (Lindblad-Toh et al. 2005). Because of their recent history, wolf and dog can produce viable offspring in the wild. DNA analyses were carried out to obtain a sound taxonomic identification. For this purpose, DNA of the dead animal was compared with DNA from European wolves, dogs and wolf-dog hybrids, using a suit of genetic analyses. A first analysis consisted of Sanger sequencing of two overlapping fragments of the maternally inherited mitochondrial (mtDNA) displacement (D-)loop control region: one of 257 base pairs (bp) (Pilot et al. 2010) and one of 500 bp (Randi et al. 2000). This was done to reveal more about the taxonomic identity of the mother.

To investigate the taxonomic identity of the father, additional analyses were carried out with biparentally inherited nuclear microsatellites (Ostrander et al. 1993, Fredholm & Wintero 1995, Francisco et al. 1996) that have been used earlier to differentiate among wolves, dogs and wolf-dog hybrids (e.g. Caniglia et al. 2013). Two independent analyses were performed, using a similar set of markers (four shared loci FH2088, FH2096, FH2137, CPH5 from Fredholm & Wintero (1995) and Francisco et al. (1996)) but a different set of European wolves and dogs as references. Scores were visualised by a Principal Coordinates Analysis (PCA).

The first reference set (C09.250, C20.253, CPH2, CPH4, CPH8, CPH12, FH2004 and FH2079 from Ostrander et al. (1993), Fredholm & Wintero (1995) and Francisco et al. (1996), in addition to the four shared loci) contained profiles from domestic dogs (n=75; 63 crossbred dogs sampled in villages in the northern Apennines and 12 German Shepherd dogs) and three wolf reference populations (n=115; 79 animals from peninsular Italy, 10 from the Carpathian mountains and 26 from the Balkans). The second reference set (FH2001, FH2010, FH2017, FH2087L, FH2097, FH2140, vWF, FH2054, FH2161, PEZ17, DBX6 and DBX7 from Seddon et al. (2005), Shibuya et al. (1994), Fredholm & Wintero (1995), Francisco et al. (1996) and Cho (2005)), in addition to the four shared loci) contained profiles from domestic dogs (n=12), wolves from Germany and western Poland (n=49) and wolves from peninsular Italy (n=15).

Isotopes - Isotope analyses of strontium ($^{87}Sr/^{86}Sr$) were carried out on bone, hair and tooth enamel of the dead wolf and beaver bone remains in its stomach. These techniques have been developed for application in human and wildlife forensic applications to determine the origin and movement of suspects and animals (Bowen et al. 2005, Hobson & Wassenaar 2008). They could be applied to this particular wildlife forensic case as strontium isotope ($^{87}Sr/^{86}Sr$) values are highly correlated with the geology of particular areas (Evans et al. 2010, Voerkelius et al. 2010; figure 7C). Teeth enamel typically records the isotope signature of an animal's early life, while hair reflects the later life stages.

Results

Macroscopy

External investigations - The dead animal was a subadult, sexually mature female with a light-coloured coat (figures 1-2), indicating a European origin (Heptner & Naumov 1998, Okarma 2000). The dead animal had traces of a supracaudal gland ('violet gland'), which is lacking in most breeds of dog (Lloyd 1980). The tail was furry, as is characteristic for wolves, and the nails were quite long, which is suggestive of an outdoor life. Coat color and pattern resembled those of European wolves in all respects. No chip or visible remains of a collar could be found. Hairs on the back, shoulders, flanks and neck were ca. 50, 53, 57-29, and 82 mm long, respectively, and not in moulting stage. Hairs on the back of wolves from the Czech Republic and the Soviet Union are reported to range from 50-85 mm in winter (Peters 1993), indicating that the dead wolf was still in winter coat. The incisors showed slight wear on the sharp edges of the lobes, the canines had slight wear on the distal end of the posterior ridge, and the carnassials did not show any visible wear. This is all indicative of an age of at least 1.5 years and at most 2.5 years old (Gipson et al. 2000). Micro-CT scans of the right carnassial (figure 3) and microscopic inspection of thin slices of the root (Grue & Jensen 1979) confirmed that the animal was just over one year old when it died. This explains why it was not molting as this is often postponed in young animals. The stomach contained hairs, a spinal disc and part of the tail of a juvenile beaver (*Castor fiber*; figure 4), a regular part of the diet of wolves in central and northern Europe (Milne et al. 1989, Andersone & Ozolins 2004). The diameter of the spinal disc was 15 mm which corresponds with a first summer individual (Rosell et al. 2010). The dimensions of the scales on the tail were 8-9 by 3-4 mm, respectively (figure 4). Scales on the tails of beavers older than a year range from 11-15 by 5-6 mm of individuals deposited in the collection of Naturalis Biodiversity Center. Beavers remain in their natal territory until they are at least 20 months old (Campbell-Parker & Rosell 2013), thus this beaver was most likely consumed in close proximity to its parental lodge.

A total of three large scats (six subsamples) were found within less than 15 km from the dead animal on two different localities in the Kuinderbos (RD-coordinates 181.5 - 534) in July 2013. The scats were deposited in the middle of a path. Shape and size (length of 20 cm and diameter of ca. 4 cm) of the scats were very different from most fox scats. Morphological analyses based on cuticular hair patterns (Teerink 1991) showed that these scats contained hairs with a similar pattern to hairs of red deer (*Cervus elaphus*). The hairs were too short for an adult so originated from a juvenile or a domestically kept deer. Morphological identification keys are unsuitable for distinguishing juveniles from domestically kept deer. One of the scats not only contained hairs but also some bone and skin fragments, an ixodid tick, and a deer throat bot fly larva (*Pharyngomyia picta*). The hairs attached to the bone and skin fragments were identified as red fox (*Vulpes vulpes*). Eggs of the nematode parasite *Eucoleus aerophilus* were found in one of the scats. This worm belongs to the Capillariidae (Nematoda, Enoplida) and is often observed in the lungs of foxes and wolves in Europe. Eggs of *Spirometra* and *Alaria,* found in the intestines of the dead wolf (see below) were not observed in the scats.

Internal investigations - The animal was in excellent condition and only carried ripe proglottids of the tapeworm *Spirometra* sp. (Cestoda, Pseudophyllidea, Diphyllobothriidae) and the trematode parasite *Alaria alata* (Trematoda, Digenea, Diplostomatidae). This is indicative of a life in the wild. *Post mortem* analysis resulted in the discovery of three bullet holes in the hide, one of 4.5 mm in the flank, and two of 6 and 7 mm diameter in the chest (figure 5). A clear bullet trace was found in the tissue of the chest, in combination with multi-

Figure 3. Micro-CT-scan of the right carnassial of the dead wolf. The number of cementum annuli (indicated by the white arrows) corresponds with a second summer individual. *Photo: Dirk van der Marel.*

Figure 5. Bullet hole (indicated by the white arrow) in the middle of a dark colored bloodstain on the inner side of the hide originally enveloping the left chest of the dead wolf. *Photo: Marja Kik.*

Figure 4. Beaver remains from the stomach of the dead wolf. *Photo: Kevin Beentjes.*

Figure 6. Re-assembled skull of the dead wolf showing multiple fractures. *Photo: Barbara Gravendeel.*

ple fragments of a copper-coated lead expanding bullet in the tissue of the animal's flank, indicating that the animal was shot twice. The dead animal had a fractured skull (figure 6), damages to the skin of the face (30 x 13 mm) and neck (76 x 14 mm), internal bleedings in the brain and thorax, torn muscles along the spine, and several damaged spinal discs, all indicative of a collision by a car or heavy beating.

Biochemistry

DNA analyses - The mtDNA sequences obtained showed a 100% match with haplotype W1, commonly found in European wolves (Pilot et al. 2010, Baltrunaite et al. 2013; areas indicated in blue and red in figure 7A). As mtDNA is maternally inherited in wolves, this confirmed that at least the mother of the dead animal had been a wolf.

The PCA plot of the first set of microsatellite markers analysed (figure 8) shows that refer-

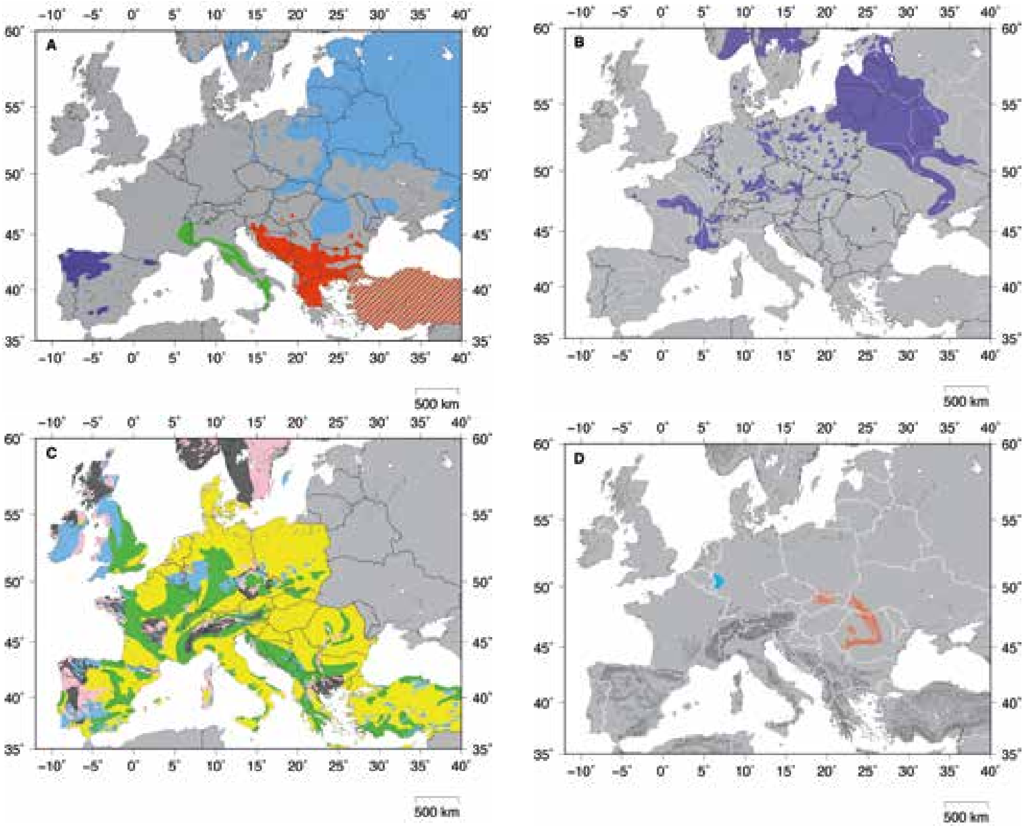

Figure 7.
A. Distribution of wolf (*Canis lupus*) haplotypes (after Pilot et al. (2010), references in the appendix and Czarnoska et al. (2013)). Blue: areas where wolves have been found with the haplotype of the dead animal (blue dashed = no data); red: areas where a southeastern European haplotype is predominant but where a northern haplotype could occur as well (red dashed = no data); green: haplotype of populations in Italy and the French Alps where the haplotype of the dead animal does not occur; purple: haplotype of Spanish and Portugese populations where the haplotype of the dead animal does not occur either.
B. Distribution of beaver (*Castor fiber*) in Europe. The beaver retrieved from the stomach of the dead wolf had an eastern haplotype which occurs over large parts of Europe (Nowak, unpublished data).
C. $^{87}Sr/^{86}Sr$ isotope values currently known from Europe (after Evans et al. (2010) and Voerkelius et al. (2010)). Yellow: 0.70701 – 0.70900; green: 0.70901 – 0.71100; blue: 0.71101 – 0.71300; pink; 0.71301 – 0.72000; grey: 0.72001 – 0.78000.
D. Distribution of main European mountainous regions indicated in dark grey; the Carpathian mountains are indicated in red, the Eifel in blue. These regions are the only areas where the haplotypes of the wolf and $^{87}Sr/^{86}Sr$ isotopes retrieved from the dead wolf and the beaver in its stomach co-occur.

ence dogs and wolves plot separately. The dead animal is included within the Carpathian and Croatian reference wolf cluster. In line with the first analysis, in the PCA plot of the second microsatellite analysis (figure 9) the dogs are also clearly separated from the wolves and the dead animal is plotted amongst wolves. In conclusion, all assignment procedures led to the identification of the dead animal as a purebred wolf.

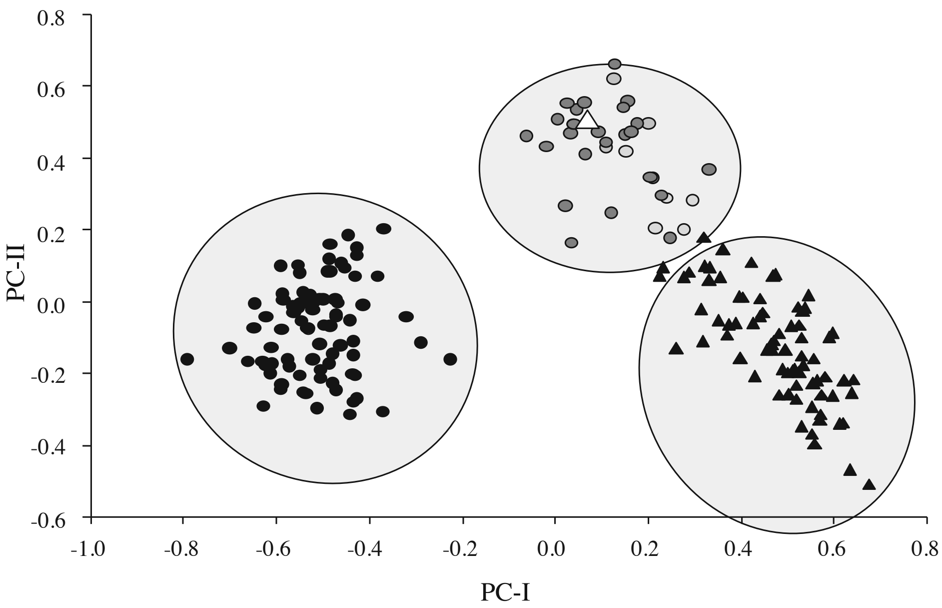

Figure 8. Principal Coordinates Analysis (PCA) plot of individual multilocus scores. Reference dogs (n=75; black triangles) and wolves (n=115; black and grey circles and white triangle) plot separately. Italian wolves (n=79; black circles) split apart from Carpathian (n=10; light grey circles) and Croatian wolves (n=26; dark grey circles). The dead animal (white triangle) is included within the Carpathian and Croatian wolf cluster.

Table 1. Details of the isotope analyses carried out on tissue samples of the dead wolf found near Luttelgeest, beaver remains found in its stomach, and three beavers from the Netherlands, Germany and the Czech Republic deposited in Naturalis Biodiversity Center (RGM) and Natural History Museum Rotterdam (NMR), respectively.

Species	**Origin**	**$^{87}Sr/^{86}Sr$ ± 2 standard error**
Beaver (RMNH.5000174)	In stomach of Luttelgeest wolf, 2013 Natural prey	0.716886 ± 0.000008 Spinal disc
Beaver (NMR9990-00842)	Czech Republic, 1992 Road kill	0.715446 ± 0.000010 Tooth
Beaver (58002CATB)	Germany, Bonn Year and cause of death unknown	0.708745 ± 0.000015 Tooth
Beaver (RMNH.MAM.55031.a)	The Netherlands, 2012, Arnhem, Drielsedijk Road kill	0.709006 ± 0.000009 Tooth
Wolf (RMHN.MAM.5000081)	The Netherlands, 2013, found along road near Luttelgeest	0.713420 ± 0.000011 Tooth 0.709033 ± 0.000012 Undercoat hair 0.709546 ± 0.000016 Guard hair

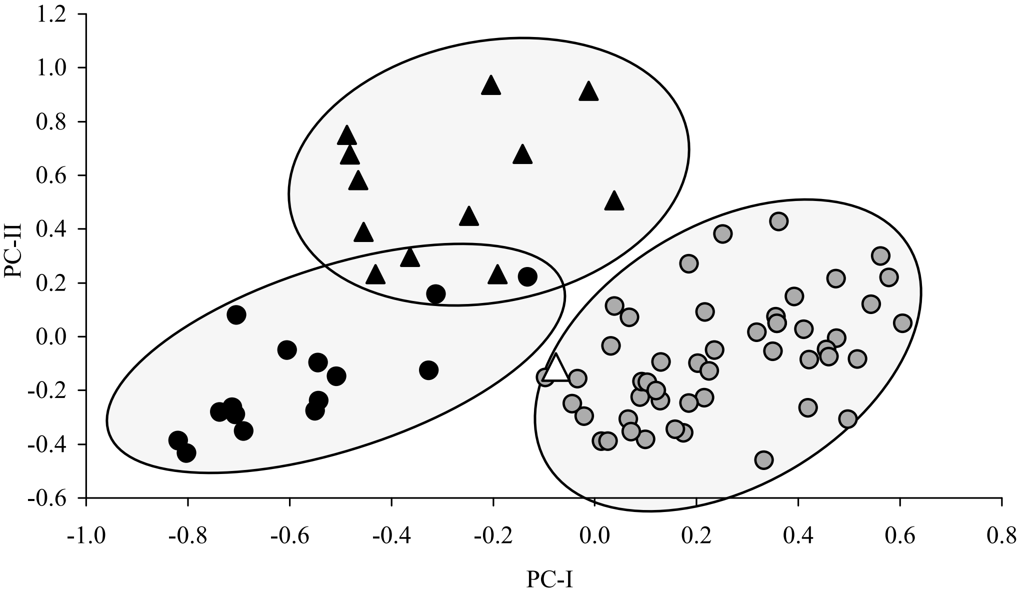

Figure 9. Principal Coordinates Analysis (PCA) plot of individual multilocus scores. Reference dogs (*n*=12; black triangles) and wolves (*n*=64; black and white circles and white triangle) plot almost separately. Italian wolves (*n*=15; black circles) split apart from German/West Polish wolves (*n*=49; dark grey circles). The dead animal (white triangle) is plotted on the outer edge of the cluster of German/West Polish wolves.

DNA sequences from the hypervariable mitochondrial control region retrieved from the beaver tail using primers employed by Durka et al. (2005) showed that this animal had an origin from eastern Europe (figure 10) or Germany (C. Nowak, unpublished data).

Genetic analyses were also performed to verify the producer of the scats analysed. DNA barcoding based on the same mitochondrial DNA fragment as used for taxonomic identification of the dead animal (Pilot et al. 2010) resulted in a DNA sequence that could be matched with red fox. As prey remains in the scat were morphologically identified as red fox, this DNA sequence may have been derived from the prey rather than the predator. In addition to the mitochondrial DNA, a total of seven loci (FH2004, FH2088, FH2137, CPH2, CPH12, FH2132 and U250 of Francisco et al. (1996), Fredholm & Wintero (1995) and Ostrander et al. (1993)) of the nuclear microsatellites produced from the dead animal could be amplified from the scats. These were all different from the alleles obtained from the dead animal (figure 11).

Isotopes - Values of $^{87}Sr/^{86}Sr$ of the dead animal's tooth enamel (0.713420 $\pm$ 0.000011; table 1) were well out of range of typical Dutch environmental isotope values (0.7085 - 0.7100; Font et al. 2012), suggesting that this animal was not born in the Netherlands. The hairs of the dead animal (both guard and undercoat hair), representing the last year of its life, had much lower values, which are in range of Dutch environmental ratios (table 1). The isotope ratios from the spinal disc of the beaver found in the wolf's stomach had the highest value of all (0.716886 $\pm$ 0.000008; table 1).

Discussion

Geographical origin - Currently, the packs of wolves occurring closest to the Netherlands

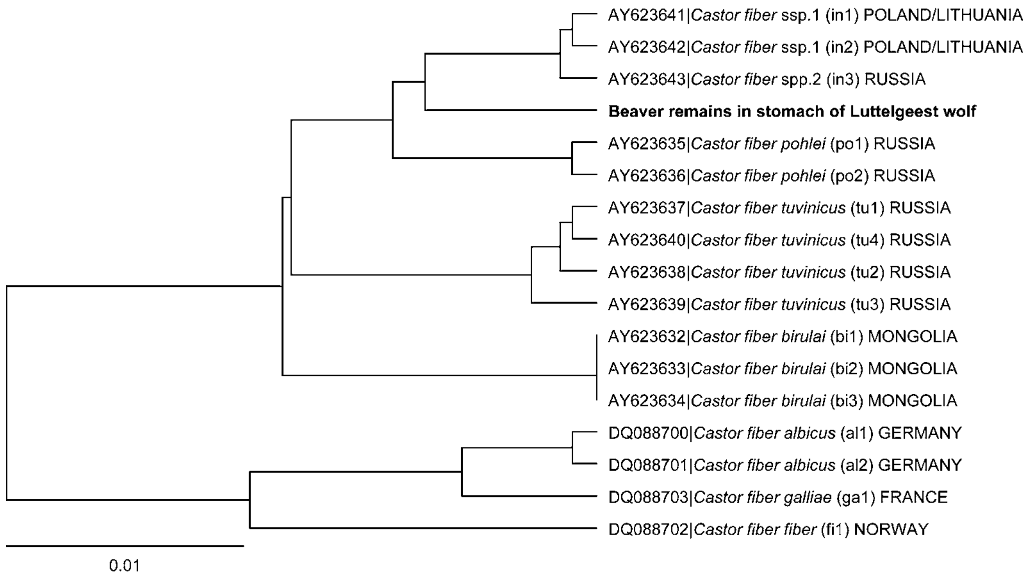

Figure 10. Unweighted Pair Group Mean Average (UPGMA) diagram of beaver (*Castor fiber*) mitochondrial Control Region haplotypes analysed. The remains of the beaver found in the stomach of the Luttelgeest wolf cluster with eastern European haplotypes.

live in northwestern Germany (federal state of Lower Saxony) (Groot Bruinderink et al. 2012). They belong to a larger population of wolves scattered mainly over eastern Germany and western Poland. Past sightings of wolves near the Netherlands were assumed to originate from this population, which Czarnomska et al. (2013) report to form the expanding western edge of a larger northeastern European population.

The mtDNA sequence obtained from the dead animal indeed fully matched with the most frequent haplotype occurring in the populations of eastern Germany and western Poland (haplotype W1 from Pilot et al. (2010)). Yet, in line with the hypothesis of a vast northeastern European population of wolves, Pilot et al. (2010) report the same haplotype to occur also (but less predominantly) throughout Fenno-Scandinavia and the Baltic States as well as in Belarus and Russia and the Ukrainian part of the Carpathian mountains (figure 7A). It is not unlikely that the same haplotype will also incidentally occur further south. Thus, based on its mtDNA data, the dead animal could have originated from an area ranging from Fenno-Scandinavia in the north up to south-eastern Europe.

Microsatellite markers typically show larger variation among individuals and populations. Unfortunately, at the time of this study no single reference set was available that included wolves from all over Europe. Two separate analyses were therefore performed to cover as many potential populations of origin as possible. The Principal Coordinates Analysis for one reference set, including Italian and German/West-Polish wolves, clearly separated populations from each other. The dead animal clustered weakly with the latter, as it is plotted at the outer edge of the cluster. Moreover, the dead animal showed several unique alleles, which have never been found in the German/West Polish population (C. Nowak, unpublished data). This makes it highly unlikely that the dead animal originated from this population. Czarnomska et al. (2013) also found individuals with unique alleles amongst the wolves sampled in western Poland, and suggested that these individuals

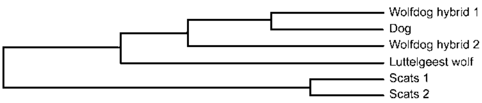

Figure 11. Unweighted Pair Group Mean Average (UPGMA) diagram of data obtained from seven nuclear microsatellites produced from the dead animal that could also be amplified from the scats found in the Kuinderbos. The data obtained show that none of the scats were produced by the dead animal.

represent immigrants from areas not covered in their analysis. Most likely it concerned immigrants from the northeast (Baltic States, Belarus, Russia), where larger populations occur that probably also contain a larger, but yet unmapped, genetic variation (Czarnomska et al. 2013). An analysis based on the other reference set used in our study suggests a different scenario. According to the other analyses carried out with microsatellites, the dead animal was included right in the middle of a Carpathian/Croatian cluster of wild wolves. A micro-array analysis including reference sets of all wolf populations occurring throughout Europe might shed further light on the origin of the dead animal.

Most recent history - The microsatellite differences found between the scats and the dead animal are sufficiently large to conclude that these cannot be linked (figure 11). Possible alternative producers were another wolf, a dog, a fox, or a wolf-dog hybrid. The fact that the scats contain red deer and red fox remains does not necessarily mean that only a wolf could have been the predator. Owners of wolf-dog hybrids commonly feed their pets locally shot deer or road kills and walk them in nature reserves where they are allowed to defecate freely. Dogs in the Netherlands are often walked off the leash and annually kill a lot of deer and other wildlife.

The isotope values obtained from the beaver remains found in the stomach of the dead animal strongly suggest that the wolf's last meal originated from a high $^{87}Sr/^{86}Sr$ catchment area. Such areas can be found in the Alps, the Carpathians, around some mineral springs originating from deep layers in the Eifel, the Pyrenees and in several smaller catchment areas in Europe (Voerkelius et al. 2010; figure 7C). All of these areas are mountainous, and too far away for the wolf to have walked to the Noordoostpolder in the Netherlands before digestion of its stomach contents. The remarkably large $^{87}Sr/^{86}Sr$ differences between the dead animal's tooth, hair and last meal suggest that it had roamed an area with significant regional $^{87}Sr/^{86}Sr$ variation. This would be in good (but not exclusive) agreement with geologically diverse areas such as the Eifel or the Carpathian mountains (figure 7D). This finding is in accordance with the eastern European haplotype derived from the tail tissue of the beaver retrieved from the stomach of the dead wolf (C. Nowak, unpublished data).

Beavers occur in large numbers in the northwestern part of the Carpathian mountains up to 500 m altitude due to successful reintroductions of Russian stock in the late 1970s in Poland (Carpathian Ecoregion Initiative 2001, Deinet et al. 2013) and in smaller numbers in the Eifel (figure 7B). The beaver was reintroduced in the latter region in the late 1980s with animals obtained from a farm in Popielno in Poland that imported its animals from Woronezj in Russia (Dalbeck et al. 2008). The clustering of the haplotype of the beaver remains retrieved from the stomach of the dead animal with Lithuanian/Polish/Russian haplotypes (figure 10) should therefore be interpretated with care as this animal could have been consumed in the Eifel. No $^{87}Sr/^{86}Sr$ isotopes studies have yet been carried out with beavers and we only sampled four specimens (table 1). Studies carried out with a larger number of replicates of different European deer species from various localities in Germany and the UK (Kierdorf et al. 2008, Sykes et al. 2006), however, showed that $^{87}Sr/^{86}Sr$ values obtained from the teeth of these herbivore species strongly correlated with the ranges of values known for different geological areas. Strontium isotope values seem therefore very useful for assessing the provenance of animals of unknown geographical origin similar to humans for which this method has already been established (Bowen et al. 2005, Hobson & Wassenaar 2008). Ongoing analyses of water samples collected in December 2013 around beaver dens in the Eifel for isotope values of $^{87}Sr/^{86}Sr$ and $^{206}Pb/^{207}Pb$ will hopefully shed further light on the geographic origin of the consumed beaver. These forensic markers are often used in combination to improve accuracy of assessing provenances (Kierdorf et al. 2008).

Migration distances of 1000 km are not uncommon for wolves as illustrated by the radio collared young German wolf 'Alan' that migrated from Germany to Belarus over circa 1000 km from start to end between 23 April and 26 September 2009, covering 1500 km in total (Schede et al. 2010). Wolves can cover great distances by running for hours at a rate of 8-9 $km.hr^{-1}$ (Mech & Boitani 2003). The dead animal could never have covered the distance of several hundred kilometres between the Eifel (where there is as yet no wolf population) or the Carpathian mountains and the Noordoostpolder in the ca. 24 hours needed to digest a juvenile beaver (Kelly & Garton 1997, Andersone & Ozolins 2004, Fuller et al. 2011). It was therefore most probably shot first and illegally transported to the Netherlands shortly afterwards.

Wildlife crime - All evidence suggests that a road accident was staged. This is, amongst others, known from the UK where farmers intentionally drive over badgers previously killed by shooting, snaring or poisoning because of a suspected infection with bovine TB (*Mycobacterium bovis*) to wipe out evidence of wildlife crime (BBC News 2012). We suspect a road kill might have been staged here as well for several reasons. First of all, no reports were made to the local authorities of a road accident in the Noordoostpolder that involved this particular animal. Secondly, the *post mortem* interval (i.e. the time that had elapsed between death and the discovery of the carcass) did not correspond with the time of *rigor mortis* (i.e. chemical processes in the muscles after death causing stiff limbs). The *post mortem* interval could never have been longer than twelve hours as the Uiterdijkenweg is a busy local road frequented by many commuters that claimed not having seen anything abnormal in the evening prior to the discovery of the carcass. The *rigor mortis* interval is estimated to have lasted at least 24 hours (DiNicola 2010). The dead animal lacked clear signs of *rigor mortis* when found in the morning of July 4th so this phase had passed. This means that the animal was moved from somewhere else after it was shot and run over or beaten up. The order of these events could not be deduced. No traces of tissue damage from freezing were observed during autopsy so the dead animal was not frozen prior to transport. As no clear signs of tissue decay were observed, the animal could not have been killed longer than two days prior to discovery (DiNicola 2010).

Media attention - Triggered by the coverage in the popular media, several people reported sightings to Staatsbosbeheer in the time period just before the dead animal was found. A putative wolf was seen in the Achterhoek in the eastern part of the Netherlands on the 8th of June about 80 km from where the dead animal was found. The animal chased a roe deer (*Capreolus capreolus*) at high speed for at least a hundred metres. Between June 15th and 18th three people reported having seen a living wolf near the Noorderring or Noordermidden road, just a few km north and west from Emmeloord in the middle of the Noordoostpolder, which is at approximately 20 km distance from where the dead animal was found. A woman reported to have seen a wolf when traveling by train from Amsterdam to Almere. Upon disembarking she verified her observation with the engineer who confirmed seeing the same animal. A local farmer saw a wolf-like animal on the land of his neighbour in the Noordoostpolder and on his own fields later that day at only 15 m distance. Another farmer close by found wolf-like prints on his land during the period mentioned above. These observations fit in the usual pattern of wolf sightings and this study underlines how cautiously such reports need to be treated.

Obviously, these observations cannot possibly be from the animal found by the roadside between Luttelgeest and Marknesse, as this individual was still living elsewhere in Europe at that time. The sightings are all classified as so-called C3 observations according to internationally agreed criteria (Kaczensky et al. 2009), as they were not from an experienced person (C2 category) or accompanied by movies or photographs or DNA evidence from hairs, saliva samples, or scats (C1 category). The behaviour displayed by the observed animal(s) does certainly raise questions too. A solitary wolf in search of a territory or mate is usually very shy. It is possible that the observations were of a dog or dog-wolf hybrid. Another explanation could be that the observations were of another wolf or wolves. There is some anecdotal evidence that young wolves display curious rather than shy behaviour when confronted with humans. A military recruit in Germany was followed by three young wolves over a substantial distance in September 2012 (http://www.ndr.de/regional/niedersachsen/heide/woelfe241.html). Whether dispersing wolves, which are far older than these young animals, are still apt to display curious rather than shy behaviour is as yet unclear. In October 2012, multiple photographs were made of a wild wolf roaming the city of Uppsala in Sweden for an entire week (Radio Sweden 2012), indicating that wolves do not always avoid densely populated areas. A young wolf walked a distance of more than 700 km from Oslo in Norway to Karlskrona in Sweden in full daylight, passing villages and cities while people were passing by, all ignored by the wolf. Many photographs bear witness of this exceptional journey (Bovée 2013). Sightings of wolves in Germany are not uncommon, but usually occur around dusk or dawn, or in areas where wolves do not expect to encounter people.

Wolf management - The discovery of this dead animal in the Netherlands accelerated compilation of a special wolf management plan demanded by the Dutch government, specifying how to deal with the re-appearance of the wolf (Groot Bruinderink et al., in press).

Conclusions

Combined macroscopic and biochemical analyses of the dead canid found near Luttelgeest on July 4th 2013 showed that it was a purebred female wolf, just over one year old, of eastern European origin. Remains of shattered bullet fragments found in the chest and flank, and a discrepancy between the timing of the *post mortem* and *rigor mortis* intervals indicate that this wolf was shot prior to its appearance in the Netherlands. The carcass was still relatively fresh, thus it must have been driven from a distance that could be bridged within two days. It fed on beaver, most likely in either the Carpathian mountains or the Eifel, before it was shot and subsequently illegally transported to the Netherlands. These areas are the only regions where the haplotypes and $^{87}Sr/^{86}Sr$ isotopes retrieved from the dead wolf and the beaver remains found in its stomach co-occur. Ongoing analyses of additional $^{87}Sr/^{86}Sr$ and also $^{206}Pb/^{207}Pb$ isotopes will hopefully shed more light on the geographical region where the beaver was consumed. (Un)intentional illegal introductions of wildlife in the Netherlands are quite common (van Diepenbeek 2006, van der Hagen 2008). Detailed morphological, molecular and isotope analyses of the carcass and the stomach contents of the wolf helped to distinguish artificial from natural dispersal here, similar to earlier cases (Gravendeel et al. 2011). We advocate regular application of wildlife forensic techniques when formerly extinct species re-appear.

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Samenvatting

De eerste in Nederland gevonden wolf sinds 150 jaar was het slachtoffer van milieucriminaliteit

De laatste wilde wolven (*Canis lupus*) in Nederland werden in 1869 waargenomen in Schinveld. Ruim 125 jaar later, op 4 juli 2013, werd een dood subadult vrouwtje gevonden op de weg tussen Luttelgeest en Marknesse in de Noordoostpolder. DNA-analyse wees uit dat het om een raszuivere wolf ging van Oost-Europese afkomst. Het laatst genoten maal bestond uit een bever die alleen in de Eifel of de Karpaten kan

zijn gevangen omdat dit de enige gebieden zijn waar $^{87}Sr/^{86}Sr$ isotopen en haplotypes van zowel de wolf als bever met elkaar overeenkomen. Beide gebieden liggen voor een wolf te ver van de Noordoostpolder vandaan om binnen een dag naar toe te kunnen lopen. Kogelfragmenten en slagsporen in de borstholte en flank, en een discrepantie in de periodes van lijkschouwing en lijkstijfheid, wijzen op afschot gevolgd door illegaal transport naar Nederland. Van natuurlijke remigratie was dan ook geen sprake.

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