



Scent marking in wolves *Canis lupus* inhabiting managed lowland forests in Poland

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Abstract

In wolves *Canis lupus*, scent marking plays an important role in territory defence. In Europe, studies on patterns of scent marking in wolves have mostly been conducted in mountains or primeval forests, but since these areas are characterised by low human activity, the impact of people on this behaviour has been neglected. We conducted a study that combined genetic methods with an analysis of the spatial distribution of wolf territory markings in lowland managed forests with high human activity. We found that scent markings are deposited by all members of wolf family groups. Wolves most intensively marked crossroads and their vicinity, especially on roads only accessible for four-wheel drive cars. Our study provides further evidence that crossroads of forest roads play a crucial role in wolf scent marking. The results of our study may be useful during inventories of wolf populations based on collecting indirect signs of their presence or non-invasive genetic sampling.

Keywords *Canis lupus* · Scent marking · Wolves · Crossroads · Forest roads

Introduction

The wolf population went through a steep historical decline, but recently, the wolf's key ecological role has been increasingly recognized, leading to the introduction of conservation programs (Mysłajek and Nowak 2015), creation of protected areas (Diserens et al. 2017), and its reintroduction to several places (Fritts et al. 1997). As a result, wolf numbers have increased both in Europe (Chapron et al. 2014) and North America (Phillips et al. 2004). This increase in wolf populations coincides with recolonisation of landscapes dominated

and shaped by humans (Kuijper et al. 2016). Wolves are the most widely distributed large carnivore species with which humans share the landscape (Mech and Boitani 2003). Although living close to humans can provide benefits to wolves in the form of human-derived resources (Newsome et al. 2015), the fear of humans as an apex predator can negatively impact large carnivores. Risk correlated with human presence can impact wolf movements, which may limit hunting and feeding behaviour (Berger 2007; Kuijper et al. 2016; Suraci et al. 2019). Wolf breeding site selection is strongly influenced by the perceived risk toward humans (Iliopoulos et al. 2014; Sazatornil et al. 2016). In forests, wolves also avoid human presence (traffic, forestry operations) by temporarily selecting areas far from people (Theuerkauf et al. 2003). That suggests scent marking behaviour may also be altered by human presence.

Wolf conservation, including management of the areas they inhabit, requires good knowledge on their number and distribution (Boitani et al. 2015). Unfortunately, monitoring of wolves is challenging, as they live in low densities, utilize vast territories (Mysłajek et al. 2018), and young individuals disperse over large distances (Andersen et al. 2015). Modern techniques, like telemetry, enable precise assessment of population parameters, but in contrast to North America, in Europe, such tools are generally only used in short-term local studies on limited numbers of individuals (e.g. Ciucci et al.

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1997; Blanco et al. 2005; Kusak et al. 2005; Jędrzejewski et al. 2007; Mysłajek et al. 2018), with the exception of Scandinavia and Finland (Mattisson et al. 2013; Kojola et al. 2018). Different techniques give different types of information: telemetry gives information about individual space use and activity (Merrill 2002; Demma et al. 2007), while camera traps can be used to access information about pack size and space use by all family group members (Campos-Candela et al. 2017). However, it is often expensive to collect sufficient good quality data using these techniques, and in the case of telemetry, in countries where wolves are protected by law (like Poland), obtaining permissions to perform this kind of research can be time consuming. Cheaper and simpler methods of wolf monitoring at the landscape level are based on recording indirect signs of their presence, like the secretions (scats, urine) they use for olfactory communication. Sign counts have been used as an index of relative abundance of large carnivores (Llaneza et al. 2014). Molecular techniques are of prime importance in conservation genetics and behavioural ecology, and these can be added to increase the power of monitoring efforts. Studies using genetic techniques based on non-invasive sampling are becoming increasingly popular (Fabbri et al. 2007; Steinglein et al. 2010). Thus, knowledge about patterns of scent marking is crucial for monitoring efforts to be successful.

Wolves mark territories using faeces, urine and secretions from glands between the toes deposited during ground scratching (Harrington and Asa 2003). Territory marking plays an important role in territory defence, pair bonding and achieving reproductive synchrony (Peters and Mech 1975; Rothman and Mech 1979; Harrington 1981; Asa et al. 1984; Paquet and Fuller 1990; Paquet 1991; Vilà et al. 1994). Intensity of scent marking varies seasonally, and is affected by the physiological status of individuals and interactions with conspecifics (Kleiman 1966; Peters and Mech 1975; Asa et al. 1990; Zub et al. 2003). Wolves increase the probability of scent mark detection by other individuals by depositing secretions in conspicuous places (Barja et al. 2004, 2005). Previous studies, conducted in North America, have suggested that wolves primarily mark the boundaries of their territory (olfactory bowl model, Peters and Mech 1975); however, more recent findings have revealed that some of the most important parts of their territory, e.g. the vicinity of den sites, are also intensively marked (hot spot model, Zub et al. 2003; Llaneza et al. 2014).

In Europe, studies on scent marking in wolves have mostly been conducted in mountains (Vilà et al. 1994; Barja et al. 2004, 2005, 2008a, b; Martín et al. 2010; Llaneza et al. 2014) or primeval forests (Zub et al. 2003), while managed forests, the primary habitat for wolves in the Northern European Plains (Jędrzejewski et al. 2008; Huck et al. 2011; Nowak et al. 2017), have been neglected. As human presence can be the overriding factor driving wolf behaviour and space

use (Theuerkauf et al. 2003; Iliopoulos et al. 2014; Sazatornil et al. 2016; Kuijper et al. 2016; Suraci et al. 2019), we aimed to assess patterns of wolf territory marking with scats and urine in intensively managed lowland forests. Following the studies of Barja et al. (2004, 2005), we hypothesised that wolves will deposit their faeces and urine in places where they are more likely to be detected by other individuals, like at or near forest road intersections. We expect also that to maximize the effectiveness of their markings, wolves will use different types of forest road intersections, characterised by lower anthropoppression than in Barja et al. (2004). There is almost no difference between the numbers of markings left inside forests, and along roads or paths (Zub et al. 2003), so as roads are often used by wolves, we chose to survey them, focusing on their usefulness in forest management. Notably, we used a novel approach combining the traditional way of analysing scent marking behaviour with non-invasive genetic techniques to reveal the role of an individual's sex and social status in territory marking.

Methods

Study area

We conducted the study in Napiwoda-Ramuki Forest (hereinafter NRF) in northwest Poland (53°33'40"–53°14'42"N, 20°33'23"–20°56'59"E) (Fig. 1). The area lies within four Forest Districts (Niedzica, Szczytno, Przasnysz, Wielbark). The terrain (1300 km²) is flat with a few small hills reaching up to 200 m above sea level. The climate is continental, with a long persistence of snow cover (94 days/year), and average temperatures in January and July of –3.1 and 16.8 °C, respectively. The forests are commercial stands dominated by relatively young (mostly 40–80-year old) planted Scots pines *Pinus sylvestris* (85%), while wetter patches are mainly overgrown with black alder *Alnus glutinosa* (8%).

Although the ungulate community in NRF consists of four large herbivore species, including moose *Alces alces*, red deer *Cervus elaphus*, roe deer *Capreolus capreolus* and wild boar *Sus scrofa* (Borowik et al. 2013), wolves in this area mainly prey on roe deer and wild boar, with additional food items being brown hare and Eurasian beaver (Jędrzejewski et al. 2012). Wolves inhabiting NRF belong to the Baltic wolf population, which is the source of the Central European wolf population that is currently recovering in western Poland, Germany, western Czech Republic, The Netherlands and Denmark (Czarnomska et al. 2013; Szweczyk et al. 2019). The study area is occupied by three wolf family groups (Geldoń et al. 2015, K.M. Stępniań unpubl.). The area is also sparsely inhabited by the Eurasian *lynx Lynx lynx* (Niedziałkowska et al. 2006). The study area encompasses the former military training area “Muszaki”, and due to their

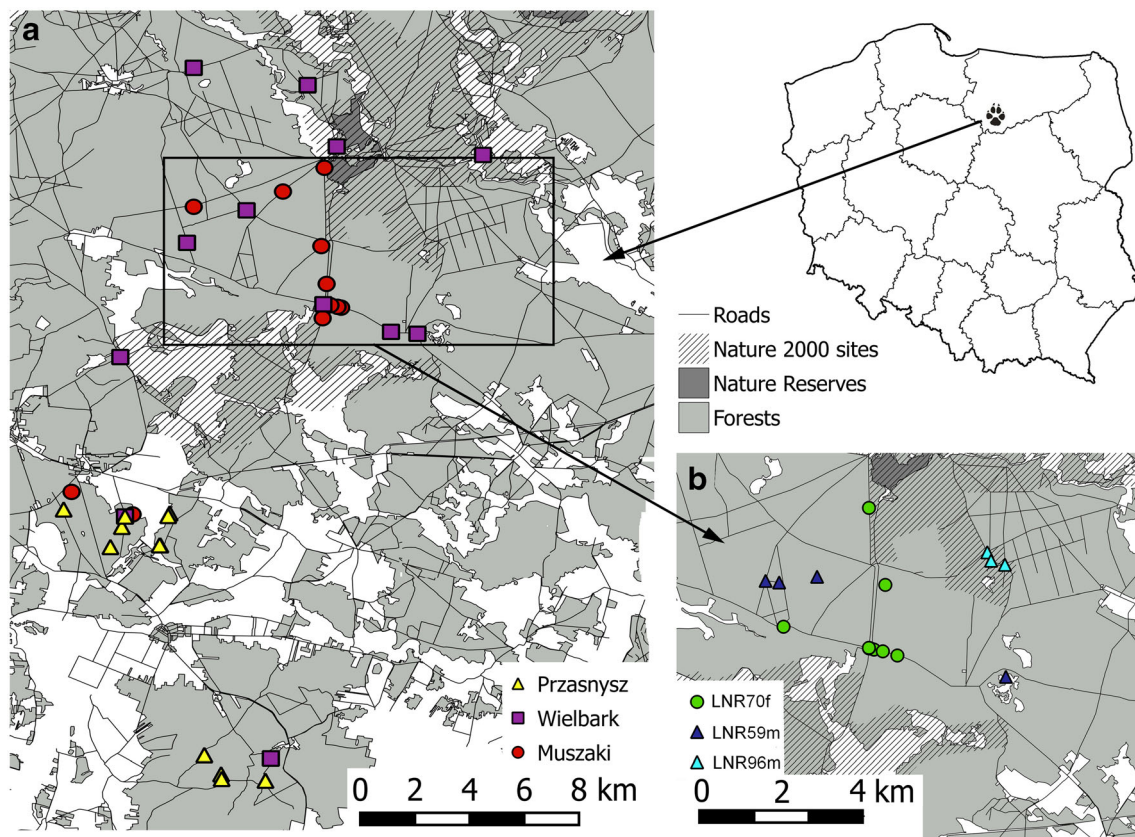


Fig. 1 Map of the study area. **a** Location of scent markings left by wolves genetically assigned to three local family groups, called Przasnysz (yellow triangles), Wielbark (violet squares) and Muszaki (red circles). **b** Location of scent markings left by wolves that were not assigned to any

family group (i.e. putative floaters), a female called LNR70f (green circles) and two males LNR59m and LNR96m (dark blue and light blue circles, respectively)

commercial use, local forests are intersected by a dense network of gravel and dirt roads. However, the use of roads administrated by State Forest Service is mainly limited to forestry operations, and public access is restricted.

Genetic analysis

We performed genetic analysis to assess the sex, numbers and genetic relationships of wolves using DNA isolated from non-invasive samples. Only samples with sufficient quality DNA (101 scats and 20 urinations) were used in these analyses. Samples were collected during the winter from December 2016 to March 2018. Due to the low number of days with snow cover in the study period, the opportunities for finding urine samples were limited.

We stored 3–4-cm fragments of fresh scats in plastic tubes (30 ml) filled with 96% ethyl alcohol, while urine was collected together with the snow on which it was left and placed into 50-ml plastic tubes with 30 ml 96% ethyl alcohol and 1.5 ml 3 M sodium acetate.

We isolated DNA from faecal samples using an Exgene™ Stool DNA mini kit (GeneAll, Korea) and from urine using an Exgene™ Genomic DNA Micro kit (GeneAll, Korea)

following the manufacturer's instructions. Each DNA extract was amplified three times in PCR reactions. To reduce the risk of contamination, we carried out DNA extractions in a room dedicated to the processing of non-invasive samples. We included negative controls in each extraction set to monitor for contamination.

We amplified the following unlinked autosomal microsatellites previously used by members of the Central European Wolf Consortium to study wolf population genetics (de Groot et al. 2016): PEZ17 (Neff et al. 1999), CPH5 (Fredholm and Wintero 1995), vWF (Shibuya et al. 1994), FH2001, FH2010, FH2017, FH2137, FH2088, FH2096, FH2097, FH2140, FH2054, FH2161 (Francisco et al. 1996). We also amplified DBX intron 6 and DBY intron 7 fragments to determine the sexes of individuals. We performed multiplex PCRs in a 10- μ l solution containing HotStarTaq Master Mix (Qiagen), each primer at 0.2 μ M concentration, 0.2 mg/ml BSA, and 3.8 μ l of DNA isolate. Amplification reactions were carried out in an MJResearch Dyad PTC-220 Peltier Thermal Cycler (BioRad) (for further details, see Lesniak et al. 2017). The products of PCR reactions were analysed on an ABI3130 DNA Analyzer (LifeTechnologies, USA) and fragment sizes were determined using PeakScanner (Applied Biosystems,

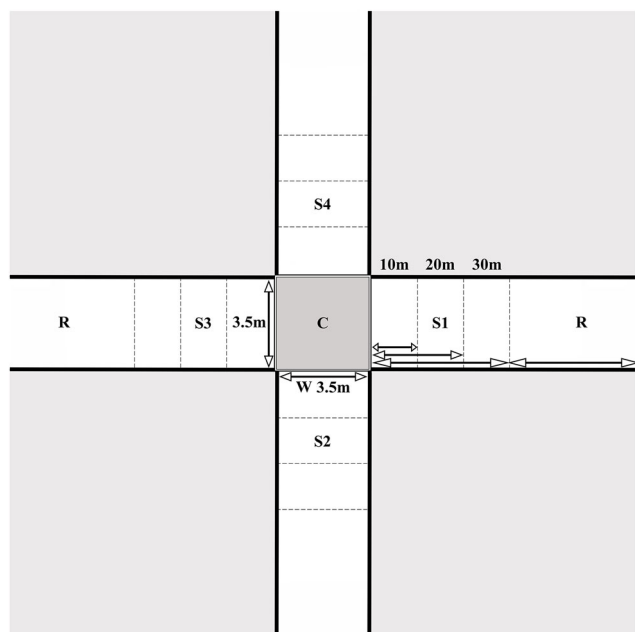


Fig. 2 Description of a forest road intersection. Grey area indicates the surface of the crossroad, lined area the surface of the periphery of the crossroad and white area roads; S_n is the number of roads leaving the crossroad

USA). Each scat sample was amplified at least three times through a multiple-tube approach (Taberlet et al. 1996). We accepted alleles confirmed by a minimum of two independent PCR amplifications, and in subsequent analyses included only individuals for which nine or more loci had been successfully amplified. We assessed relatedness of individuals using software for analysing parentage from co-dominant genetic markers such as microsatellites: Cervus (Kalinowski et al. 2007) and Colony (Jones and Wang 2010).

Assessment of scent marking patterns

We conducted studies on wolf scent marking from December 2016 to March 2017. In this period, we made 18 transects, with a total length of 110.93 km (T) and checked forest intersections 436 times (C) (Table 3). We inspected 5.34 km of crossroads, 30.56 km of peripheries of crossroads and 352.36 km of roads. When we checked the same intersection more than once, only markings deposited after our last check were considered to be new markings. The average width (W) of roads was 3.5 m. The locations of scent markings (scats and urinations) of wolves were recorded with a hand-held GPS unit (GPSMAP 64st, Garmin, USA). A description of each marking's surroundings was made using the following parameters (Fig. 2): (1) location of marking: on road, on crossroad or on periphery of crossroad; (2) distance between marking and border of intersection; (3) driveability: type 1 (high, easy crossroad)—road without obstacles, easily accessible for every type of car, type 2 (medium, medium crossroad)—road

with small obstacles that make driving harder for a two-wheel drive car, type 3 (low, difficult crossroad)—road traversable only with a four-wheel drive vehicle; and (4) type of intersection. When intersections were composed of roads with different driveabilities, they were classified by the least driveable road. We additionally divided intersections into type 1 (simple) with a maximum of three exit roads, and type 2 (multiple) with four, five or six exit roads. We assumed a “crossroad” (Fig. 2) to be a square with side lengths equal to the average width of roads. We calculated the total area of crossroads surveyed with the formula $T_c = W^2 \times \Sigma C$. The area from the border of a crossroad to 30 m away was defined as the “periphery of a crossroads”. We divided this area into three parts: 0–10 m, 11–20 m and 21–30 m from the border of the crossroad. We calculated the total area with the formula $T_s = W \times \Sigma S \times L$. The area outside crossroads and its peripheries was categorised as “road”. The formula for this calculation was $T_r = T_t - (T_c + T_s)$. We calculated the total studied area with the formula $T_t = T \times 3.5$. We always surveyed the entire area of crossroads, crossroad peripheries and roads. To facilitate comparisons, all formulas for area calculations and driveability categories are modelled on Barja et al. (2004).

Results

Wolf population within study area

We genetically identified 25 wolves within the study area: 17 males and 8 females, which left 34 and 23 markings, respectively (Table 1). We obtained 2.0 samples per male ($\chi^2 = 0.468$, $df = 1$, $P > 0.05$) and 2.9 samples per female ($\chi^2 = 1$, $df = 1$, $P > 0.05$), but there were no statistical differences between the numbers of samples and expected values. We found three highly related groups (Wielbark, Przasnysz, and Muszaki groups) and several individuals that were not related to any of the three family group members, probably floaters (Fig. 1b). From the Wielbark group, we identified seven individuals, including the breeding male and six of his offspring. From the Przasnysz group, we identified six individuals, including the breeding pair and four of their offspring. From the Muszaki group, we identified seven individuals, including the breeding female and six of her offspring (Table 1).

The largest numbers of markings were left by two individuals unrelated to either of identified groups—female LNR70F and male LNR59M (Table 2). In winter 2017, female LNR70F left 11 markings on one of the biggest roads and on one of the biggest crossroads in the study area—mostly urine with blood, which suggests that she was in heat. At the same time, LNR59M left five markings, also mostly urine, near the markings of LNR70F. We identified three more unrelated individuals—male 132M who left a scat on Przasnysz

Table 1 Number of scent markings left by individuals identified by the analysis of 13 microsatellite loci

Family	Individual	Sex	Number of m. w. scats	Number of m. w. urine
Muszaki	LNR36F*	Female	1	0
Muszaki	LNR60M	Male	1	4
Muszaki	LNR102F	Female	1	0
Muszaki	LNR144M	Male	1	0
Muszaki	LNR111M	Male	2	0
Muszaki	LNR52F	Female	1	0
Muszaki	LNR108M	Male	1	0
Total	7	3F/4M	8	4
Przasnysz	LNR46M*	Male	2	0
Przasnysz	LNR34F*	Female	1	0
Przasnysz	LNR49F	Female	2	0
Przasnysz	LNR119M	Male	2	0
Przasnysz	LNR129F	Female	3	0
Przasnysz	LNR48M	Male	2	0
Total	6	3F/3M	12	0
Wielbark	LNR69M*	Male	1	0
Wielbark	LNR151M	Male	3	0
Wielbark	LNR76M	Male	0	2
Wielbark	LNR98F	Female	3	0
Wielbark	LNR130M	Male	1	0
Wielbark	LNR49M	Male	1	0
Wielbark	LNR127M	Male	1	0
Total	7	1F/6M	10	2
unknown	LNR70F	Female	3	8
unknown	LNR59M	Male	1	4
unknown	LNR96M	Male	1	0
unknown	LNR85M	Male	0	3
unknown	LNR132M	Male	1	0
Total	5	1F/4M	6	15

A star symbol (*) denotes the most probable breeding individual. Relationship between individuals and their status in family group was determined in Cervus (Kalinowski et al. 2007) and Colony (Jones and Wang 2010) software

group's territory and two others, LNR85M and LNR96M—both marked with urine near the markings of LNR70F.

Locations of wolf markings

We inspected 436 forest intersections and only 26% ($n = 112$) of them was marked. In total we found 130 markings. Their density (n/km^2) was highest in the peripheries of crossroads (3.4 samples, $avg. = 5.61$, $SE \pm 0.19$), medium on crossroads (1.5, $avg. = 0.44$, $SE \pm 0.45$) and lowest on roads (0.15, $avg. = 1$, $SE \pm 0.10$). The total density of markings at crossroads and their peripheries (these categories were combined due to the small number of samples) was much larger ($\chi^2 = 840.1951$,

$df = 2$, $P < 0.001$) than the expected value, while the density of markings on roads was significantly lower ($\chi^2 = 83.8824$, $df = 2$, $P < 0.001$) (Table 3). We found a significantly higher number of markings than expected at multiple intersections (56 markings on 186 crossroads surveyed) ($\chi^2 = 19.185$, $df = 1$, $P < 0.001$). At simple intersection, the number of markings was lower than expected (19 markings on 252 crossroads surveyed) ($\chi^2 = 13.002$, $df = 1$, $P < 0.001$).

The most marked were intersections with medium driveability (56 markings on 213 crossroads). The frequency of markings on this type of intersection was higher than expected ($\chi^2 = 10.19$, $df = 1$, $P < 0.001$). On the other hand, at the crossroads with low driveability, the marking frequency was lower than expected ($\chi^2 = 10.42$, $df = 1$, $P < 0.001$). For the high driveability type, we found no statistical difference between the expected and observed frequencies ($\chi^2 = 0.08$, $df = 1$, $P > 0.05$). Moreover, this type of intersection was the least frequently marked (Fig. 5).

Markings were not deposited randomly. Their number decreased with distance from the border of crossroads: 65% were deposited between 1 and 10 m away from the border, 28.2% between 10 and 20 m and only 6.3% between 20 and 30 m (Fig. 3). Separately, most urine markings were between 1 and 10 m (73%) away, then 16.1% between 10 m and 20 m

Table 2 Number of markings, expected and observed frequencies of wolf markings left by related and unrelated individuals

	Number of individuals	Markings		Frequency	
		Scat	Urine	Observed	Expected
Related	20	30	6	36	45.6
Unrelated	5	6	15	21	11.4
Total	25	36	21	57	57

Table 3 Expected and observed frequencies of wolf markings in the two types of area: crossroads with their peripheries and roads

	Frequency		Kilometres Surveyed
	Observed	Expected	
Crossroads (T_c) and periphery of crossroads (T_s)	112	12.02	35.89
Roads (T_r)	18	117.98	352.36
Total (T)	130	130	388.25

and 10.9% between 20 m and 30 m. The number of scat markings also decreased with distance: 62.1% were between 1 and 10 m, 34.5% between 10 and 20 m and only 3.4% between 20 and 30 m (Fig. 4).

The 20 related individuals altogether left 36 markings (1.8 marks/individual), while the 5 unrelated individuals altogether left 21 markings (4.2 marks/individual) (Fig. 5). We found a statistical difference in marking rate between the expected and observed frequencies in related and unrelated individuals ($\chi^2 = 10.746$, $df = 1$, $P < 0.05$) (Table 2).

Discussion

Peters and Mech (1975) revealed that in wolves, territorial scent marking is done primarily by the parental pair, while other members of family groups play a minor role. Our study, however, confirms that all members of wolf family groups leave scats and urine on roads. We are aware that not all defecations and urinations are left to serve as scent markings. Nonetheless, the accumulation of scats and urine from various but related individuals is itself clear information about the strength of the group and may deter potential intruders. In aggressive interactions between neighbouring wolf family groups, the odds of a pack displacing its opponent are greatly improved by its size (Gese 2001), and groups with more old individuals or adult males have higher

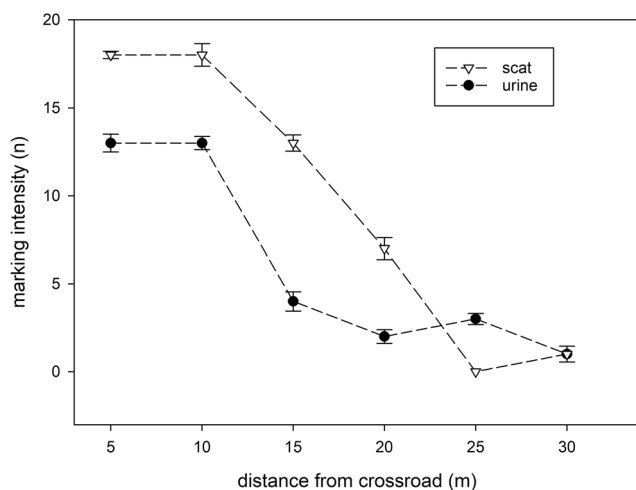


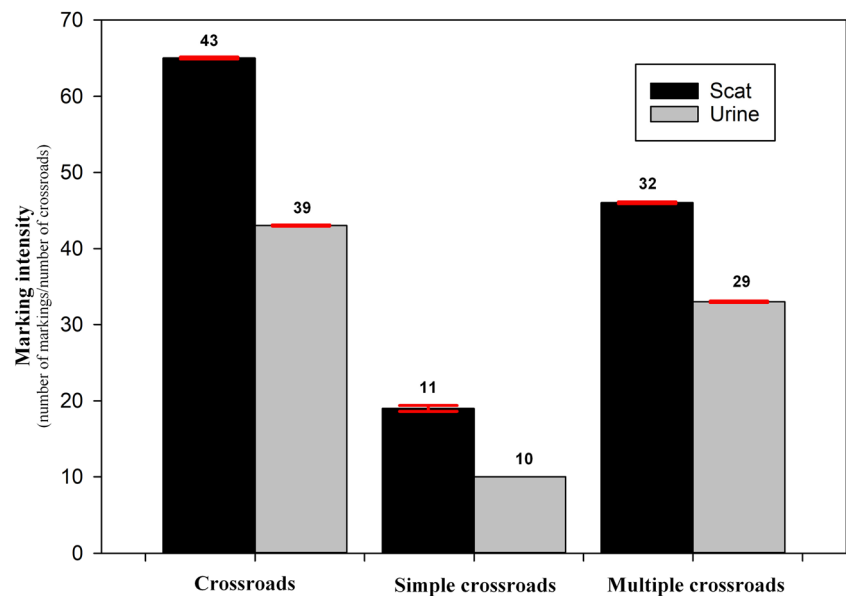
Fig. 3 Distance and density of all types of scent markings on the periphery of a crossroad

chances of winning even with a numerical disadvantage (Cassidy et al. 2015). Scent marks may indicate the identity of individuals, including information on sexual status and age (Johnson 1973); thus, intruders discovering scats and urine of various individuals may also acquire information about the number and composition of the group inhabiting a given territory, allowing them to assess their chances of winning in potential aggressive encounters.

In carnivores, the frequency of marking varies with an individual's reproductive status, being the most intensive during the mating season (Macdonald 1980). Our observations show a high frequency of marking in a female with signs of heat, i.e. a female with oestrous blood in the urine. This female was unrelated to any of the wolf family groups living in the study area, and still showed a high rate of scent markings (urine with blood), which may suggest that she was looking for a male to form a pair (Russell, Rothman and Mech 1979). At the same time, three unrelated males left markings near hers, suggesting that she may have been the reason of this behaviour. The difference between the marking rates of full residents of territories and unrelated wolves was statistically significant; however, the majority of unrelated markings were left by only two individuals. Wolf packs generally are composed of family units consisting of a mated pair and their offspring of one or more years (Mech and Boitani 2003). The individuals that did not show any genetic relationship with the identified families, we assumed to be floaters. Floating wolves may be old animals that left their former packs or young dispersing individuals trying to acquire their own territories (Mech and Boitani 2003). Nonetheless, they represent an important reproductive buffer, as they can readily replace losses of resident family breeders (Fuller et al. 2003). As a result, it is difficult to assess whether this behaviour is specific for all floaters or just for those particular individuals. Further studies are necessary to explore this issue.

Our study confirms earlier suggestions (Peters and Mech 1975; Vilà et al. 1994; Barja et al. 2004; Zub et al. 2003) that forest crossroads and their immediate vicinities play a crucial role in wolf scent marking, as in other canids (Barja et al. 2001; Barja and List 2014); however, it also highlighted the possible impact of a higher human presence on wolf behaviour in more anthropogenic landscapes. In Ramuki-Napiwoda Forest, three important factors influenced the intensity of marking: distance from crossroad, the number of roads leaving crossroads and road driveability. There was a higher chance of finding both types of

Fig. 4 Marking intensity (number of markings/number of crossroads) with scats and urine on all scent marked crossroads, simple crossroads and multiply crossroads. Means (\pm SE) are based on the number of scent markings found on each crossroad type. Number of crossroads are indicated at the top of the column

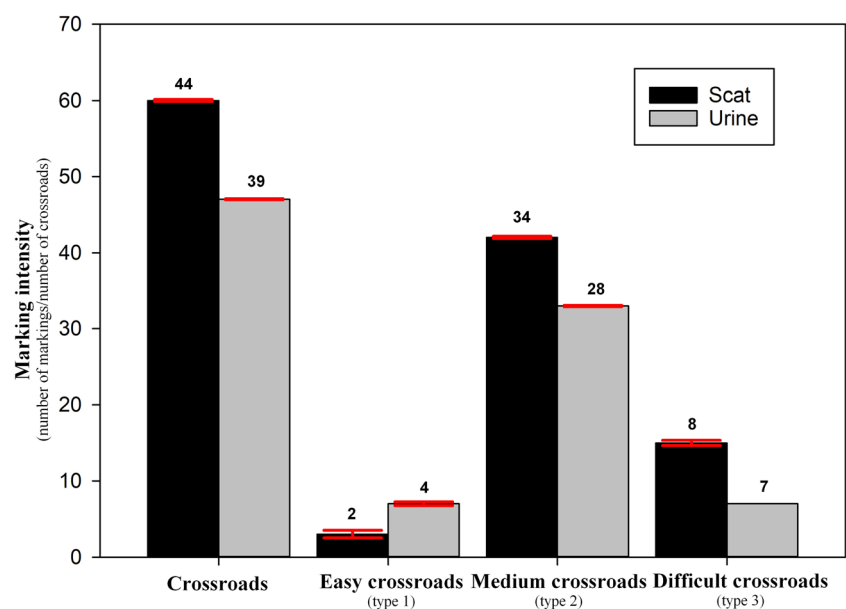


markings close (between 0 and 10 m) to crossroads, and this was true up to 20 m from a crossroad for urinations and up to 25 m for defecations. Since wolves actively search for markings as they enter crossroads (Barja et al. 2004), this relationship seems to be logical, as markings left near the joining of roads will have a higher probability of being detected than those left further away. Wolves readily use dirt roads to facilitate their movement (Bojarska et al. 2020), which increases their prey search rate (Whittington et al. 2011; Dickie et al. 2017). Thus, more intensive marking of multiple crossroads maximizes the efficiency of their olfactory communication. The rate of a marking is also dependent on the type of crossroads. Significantly more markings were found at crossroads of roads passable only with four-wheel drive cars (type 2). The higher intensity of traffic on the

better quality roads probably increases the probability markings are destroyed. This may also explain why on cross roads with high driveability (type 1) we found few scats in general and a higher frequency of urine marks compared to scats.

There are differences between the parts within a wolf territory that are most often marked between our study and study areas in Spain (Barja et al. 2004) and Bialowieza Primeval Forest (Zub et al. 2003). In Spain, the highest number of scats was left on crossroads while in Bialowieza Primeval Forest and our study, the highest marking intensity (scat and urine) occurred within the periphery of crossroad. This variation may be the result of the lower number of crossroads/km in Barja et al. (2004) study (0.12 crossroad/km) compared to 1.12 crossroad/km in our study area. Additionally, comparing the number of markings/km on

Fig. 5 Marking intensity (number of markings/number of crossroads) with scats and urine on all scent marked crossroads, type one (easy crossroads, high driveability), type 2 (medium crossroads, medium driveability) and type 3 (difficult crossroads, low driveability). Means (\pm SE) are based on number of scent markings found on each crossroads type. Number of crossroads are indicated at the top of the column



crossroads and peripheries of crossroad and roads in Spain (8.3, 1.67 and 0.15, respectively; 10.12 in total) with those observed in our study (1.5, 3.4 and 0.15, respectively, 5.05 in total) suggests that wolves studied by Barja et al. (2004) were 2 times more intensive marking than wolves inhabiting managed lowland forests in Poland. In general, the results of our study are concordant with those of Barja et al. (2004) as in both areas, wolves prefer to deposit their markings on forest road junctions or in their near vicinity. In more detail, we can find a higher deposition of markings on the periphery in contrast to Barja et al. (2004), but still close to the crossroad. This may be indicative of a behavioural adaptation of wolves that live in areas with higher human presence, where scats at the periphery may have a bigger chance to be found by other individuals and less chance of being destroyed by vehicles. On the contrary, some of our results contrast with those of Bojarska et al. (2020) who reported that a majority of scats and minority of urine markings were found on high driveability roads, with these high-traffic forest roads offering the highest probability of finding wolf scats. Therefore, it is possible that other factors, e.g. the presence of different wolf packs, landform or behavioural patterns inherited from natal family groups, are more important for wolves in maintaining territories and that human presence plays a minor role. Wolf scent marking patterns are still poorly understood, and thus, there is a necessity for further research.

Besides providing insights into patterns of territory marking, the results of our study are useful for the monitoring of wolf populations based on the collection of indirect signs of wolf presence or non-invasive genetic samples (Reinhardt et al. 2015). During field work, special attention should be paid to forest road intersections, which serve as marking hot spots. Due to the higher activity of wolves at junctions, they are also a good place for mounting camera traps. Such an approach seems to be the most cost-effective for obtaining valuable data on wolf presence in managed forests characterized by high forest road density.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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