Wolf response to two kinds of barriers in an agricultural habitat in Spain

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Abstract: We examined the effect of two kinds of barriers on an expanding gray wolf, *Canis lupus* L., 1758, population in an agricultural habitat in north-central Spain. The barriers were (*i*) a four-lane fenced highway along a flat area without wildlife-crossing facilities, and (*ii*) the River Duero Artery (RDA), comprising the river itself (50–100 m wide) and several small infrastructures along it. From March 1997 to October 2001, all 4 radio-collared wolves living <15 km from the highway (1 adult territorial male, 1 territorial breeder female, 1 dispersing male, and 1 female in 3 periods of her life (territorial immature, disperser, and territorial breeder) crossed it on between 4% and 33% of 45–163 monitoring days via vehicle bridges. Moreover, 4 more highways that we monitored in areas without radio-collared wolves have not delayed expansion of the increasing wolf population, suggesting that these highways are not an important barrier for wolves in our study area. In contrast, only 3 of 8 wolves radio-collared <5 km from the RDA were detected crossing it, and 2 of those 3 started to cross it only after severe habitat disturbance; in addition, the RDA seems to have delayed wolf expansion for some 15 years, which suggests that it is a semipermeable barrier for wolves. We discuss the likely consequences of the RDA on the recovery of the Iberian wolf population.

Résumé : Nous examinons les effets de deux types de barrières à l'expansion d'une population de loups gris, *Canis lupus* L., 1758, dans un habitat agricole dans le centre nord de l'Espagne. Les barrières sont (*i*) une route clôturée à quatre voies dans une région plane sans aménagement prévu pour la traversée des animaux sauvages et (*ii*) le tronc principal du fleuve Duero (RDA) incluant la rivière elle-même (50–100 m de large) et plusieurs petits ouvrages riverains. De mars 1997 à octobre 2001, les quatre loups munis de colliers émetteurs vivant à <15 km de la route (1 mâle adulte territorial, 1 femelle reproductrice territoriale, 1 mâle en période de dispersion et 1 femelle durant trois phases de son cycle, soit celles d'immature territoriale, de femelle en dispersion et de reproductrice territoriale) ont traversé la route entre 4 % et 33 % des 44–165 jours d'observation en passant par les ponts pour véhicules. De plus, nous avons surveillé quatre autres routes dans des régions où les loups ne portaient pas de collier et celles-ci n'ont pas entravé l'expansion des populations de loup en croissance, ce qui laisse croire que ces routes ne constituent pas des barrières sérieuses pour les loups dans notre région. En revanche, seulement 3 des 8 loups munis de colliers émetteurs vivant à <5 km du RDA ont été observés le traverser et 2 de ces 3 loups n'ont commencé à le traverser qu'à lui suite d'importantes perturbations de leur habitat. De plus, le RDA semble avoir retardé l'expansion des loups durant environ 15 ans, ce qui laisse croire qu'il forme une barrière semi-perméable pour les loups. Les conséquences probables du RDA sur la récupération de la population ibérique de loups fait l'objet d'une discussion.

[Traduit par la Rédaction]

Introduction

Gray wolves, *Canis lupus* L., 1758, are increasing in numbers in both Europe and North America and are beginning to reoccupy semi-wilderness and agricultural lands (Mech 1995; Boitani 2003). In recent decades, knowledge of wolf habitat requirements (Mladenoff et al. 1995, 1999; Mladenoff and Sickley 1998; Corsi et al. 1999; Massolo and Meriggi 1998) and dispersal (for reviews see Boyd and Plestcher 1999; Mech and Boitani 2003) has improved notably. Nevertheless, in their review on the important gaps in knowledge regarding wolf population dynamics, Fuller et al. (2003: p. 190) stated that despite the thousands of scientific articles written about wolves, we still need to know what constitutes a barrier to dispersal.

Knowledge of the impact of topographic and artificial barriers on wolves is very limited. Carmichael et al. (2001) characterized the genetic structure among gray wolf populations in northwestern Canada to ascertain whether several topographic features act as a barrier. They showed that gene flow is significantly reduced across the Mackenzie River and the Amundsen Gulf, but since these are frozen for 6–8 months of the year, they should not in themselves pose a significant barrier to wolf movement. Those authors concluded that migration patterns of caribou, *Rangifer tarandus* (L., 1758), may be the primary determinant of wolf population structure. A four-lane fenced highway in Banff National Park seems to hinder the movements of wolves (Paquet and Callaghan 1996), although crossing structures mitigate its barrier effect (Clevenger and Waltho 2000). In contrast, a

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four-lane unfenced highway in Wisconsin does not have any apparent influence on the movements of wolves (Kohn et al. 1999).

Several studies have shown that highways, railways, and large rivers can act as a barrier to other large carnivores, preventing or retarding their movements. Four-lane fenced highways limit brown bear, *Ursus arctos* L., 1758, movements in Banff National Park (Gibeau and Herrero 1998) and Slovenia (Kaczensky et al. 2003), and in Arkansas the 1600 m wide Mississippi River deflects black bear, *Ursus americanus* Pallas, 1780, movements, mainly those of females (White et al. 2000). In California, mountain lions, *Puma concolor* (L., 1771), used areas right up to the urban edge, but were often killed on roads; highways were significant filters (but not absolute barriers) to movement (Beier 1995).

Other studies have shown the capacity of carnivores to adapt to disturbed habitats or corridors where roads and highways are an important component (bobcats, *Lynx rufus* (Schreber, 1777), and coyotes, *Canis latrans* Say, 1823; Tigas et al. 2002). In addition, many studies have tested the effectiveness of crossing structures to mitigate the barrier effect of highways and railways on carnivores (Foster and Humphrey 1995; Rodríguez et al. 1997; Clevenger and Waltho 2000; Clevenger et al. 2001*b*, 2002; Gloyne and Clevenger 2001; Cain et al. 2003).

A wide obstacle and impermeability can magnify the barrier effect (Clevenger and Waltho 2000; White et al. 2000). The barrier effect can vary, not only because of barrier characteristics but also because of other factors, such as species (Clevenger and Waltho 2000; Kaczensky et al. 2003), habituation to humans (Gibeau and Herrero 1998), a population's demographic characteristics, which affect dispersal (Swenson et al. 1998; Kohn et al. 1999; Kaczensky et al. 2003), and habitat around the barrier (Clevenger and Waltho 2000; Kohn et al. 1999).

Since studying large carnivores is difficult and expensive (Minta et al. 1999) and the interaction of factors noted above can be very complex, available information is scarce and it is difficult to deduce general patterns. However, Spain's wolf population offers a good opportunity to compare the effects of different kinds of barriers and assess wolf adaptation to man-made infrastructures. In Spain, as in other southern European countries (Boitani 1982), wolves live in close contact with humans. Following centuries of severe persecution, Spanish wolves reached their lowest point circa the 1970s, when the main population was restricted to the mountains of the northwest. Subsequently, wolves were partially protected and the largest population started to increase and expanded southwards and eastwards (Blanco et al. 1992). During the 1980s, wolves appeared in flat, treeless agricultural areas and they reached the River Duero, but the population almost stagnated until the late 1990s, when several packs were detected immediately south of the river (Blanco and Cortés 2002). Since Spain joined the European Union in 1986, some 2000 km of four-lane fenced highways has been built within the 90 000-km^2 northern wolf range, and much more is planned (Ministerio de Obras Públicas, Transportes y Medio Ambiente 1994). Both highways and the River Duero Artery, comprising the River Duero and the infrastructures built along it, are thought to act as a barrier to wolves.

Our aim is to assess and compare the factors that influence the permeability of these two types of barriers on an expanding wolf population in an agricultural habitat from two perspectives: (1) the individual approach, documenting the effect of both barriers on radio-collared wolves; and (2) the population approach, documenting the effect on population expansion.

Methods

Study area

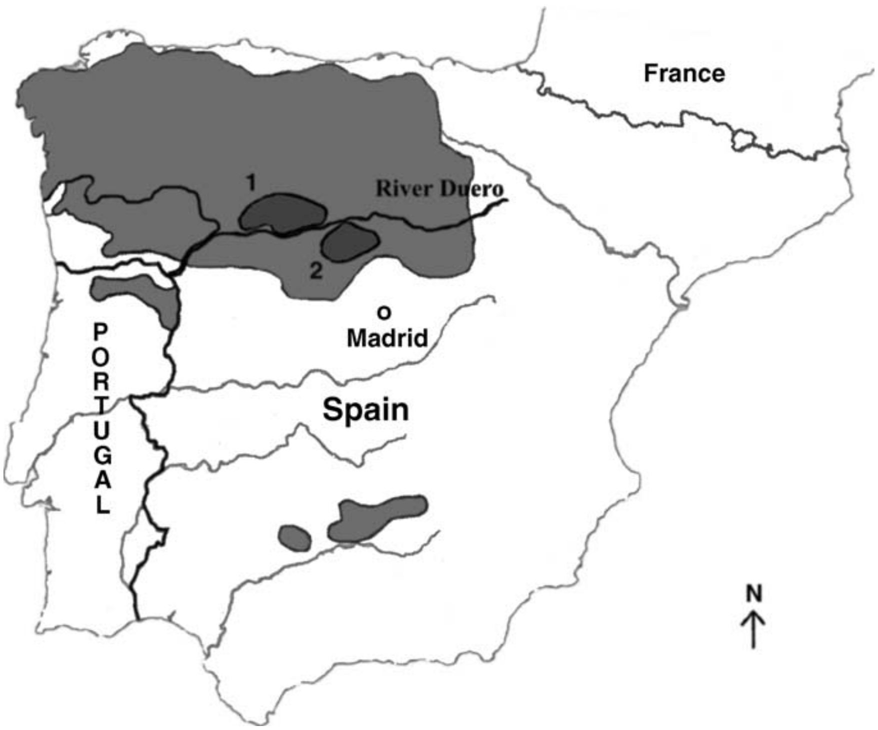
Our study was conducted in the provinces of Valladolid and Zamora in north-central Spain (Fig. 1). The study area comprises flat, almost treeless agricultural land, with cereal and maize fields that provide some cover for wolves during late spring and early summer and in late summer and autumn, respectively. Only 7% and 26% of the area is covered by remant forests north and south of the River Duero, respectively. These island forests are generally privately owned, with restricted access, and consist of evergreen oaks, *Quercus ilex* L., Portuguese oaks, *Quercus faginea* Lam., and pines (*Pinus pinaster* Soland., non Ait.and *Pinus pinea* L.). Wild boar, *Sus scrofa* L., 1758, the only wild ungulate in the area, is common in the remnant forests (around 1 individual/km^2) but almost absent in agricultural areas (Cortés 2001). European rabbits, *Oryctolagus cuniculus* (L., 1758), are locally abundant, and there are 3.5 Iberian hares, *Lepus granatensis* Rosenhauer, 1856, per square kilometre in optimal areas (Calzada and Martínez 1994). The human population (10–40 inhabitants/km^2) engages in agriculture and to a lesser extent in livestock farming. Sheep flocks are usually protected by shepherds during the daytime and locked in pens at night, so damage to livestock is moderate and people's tolerance of wolves is higher than in other parts of Spain (Blanco and Cortés 2002). North of the River Duero, wolves can be legally hunted, but with many restrictions; south of the Duero they are fully protected. Illegal persecution is common on both sides of the river (Cortés 2001).

After being absent for most of the twentieth century, wolves have been recolonizing this area since the 1970s. They live in packs of 5–10 individuals, their staple diet being livestock carrion from carrion pits. Although habituated to human activities, they fear people and avoid them by being mainly nocturnal (Vilà et al. 1995; Cortés 2001).

Barriers

We studied the influence on wolves of two kinds of barriers: (1) National Highway A-6 is a four-lane 40–60 m wide fenced highway without specific crossing facilities for wildlife or livestock. The A-6 is one of the 6 major radial Spanish highways. The fence is 1.9 m high and mesh size is 16 cm × 15 cm. It is sunk into the ground 5 m from the road. The lanes in both directions are separated by a median strip of vegetation. Our study was conducted along a 58-km segment of open, flat agricultural land, approximately between the towns of Tordesillas and Villalpando, which comprised the radio-collared wolves' home ranges. Maximum and minimum traffic volumes in January 2000 were 712 vehicles/h at 1100–1200 and 93 vehicles/h at 0500–0600 (Spanish Ministry of Transport, unpublished data). This highway crosses the River Duero in Tordesillas.

Fig. 1. Gray wolf (*Canis lupus*) range in Spain (light-shaded) (Blanco and Cortés 2002) and our study areas to the north (1) and south (2) of the River Duero (dark-shaded).



Since the highway is fenced along its length, wolves must cross it at specific points. In our study segment there are 31 asphalted bridges for vehicles over the highway (0.53 bridges/km), 14 of them (45%) connecting unpaved forest roads and 17 (55%) connecting paved roads. All bridges are 50–70 m long and 8–12 m wide with no specific adaptations for wildlife crossing. As regards human intrusion, we considered bridges in disturbed areas, i.e., ≤200 m of inhabited housing (38% of the bridges), and in undisturbed areas (62% of the bridges) >200 m from housing. In addition, there are 1.7 culverts/km that are 40–80 m long and 1 m in diameter (these are apparently unsuitable for wolves; Clevenger et al. 2001*a*), and 7 underpasses to accommodate small streams with banks <5 m wide, which may allow wildlife to cross; however, 5 of them are in, or close to, small towns. (2) The RDA comprises the River Duero itself (width 50–100 m and maximum depth 5–8 m) and one two-lane unfenced road, a railway line, two 1.5 m wide channels, and two 500 m wide unforested strips on each side of the river, with a higher density of houses and level of human use than the rest of the landscape. The artery is 1 km wide and the transport features and channels are parallel to the river. The top of the concrete channels, which run along most of the river, project 80 cm above maximum water level; their steep banks have a 50°–55° slope, but are usually slippery and difficult to climb. There are small bridges for pedestrians every 300–500 m. As regards the River Duero, we studied one 20 km long segment with 3 bridges around Toro (Zamora) and a 14 km long segment with 1 bridge around Quintanilla (Valladolid). Besides the bridges, small dams and fords (1 every 2 km, on average) allow wolves to walk or swim short distances across the river, mainly in summer.

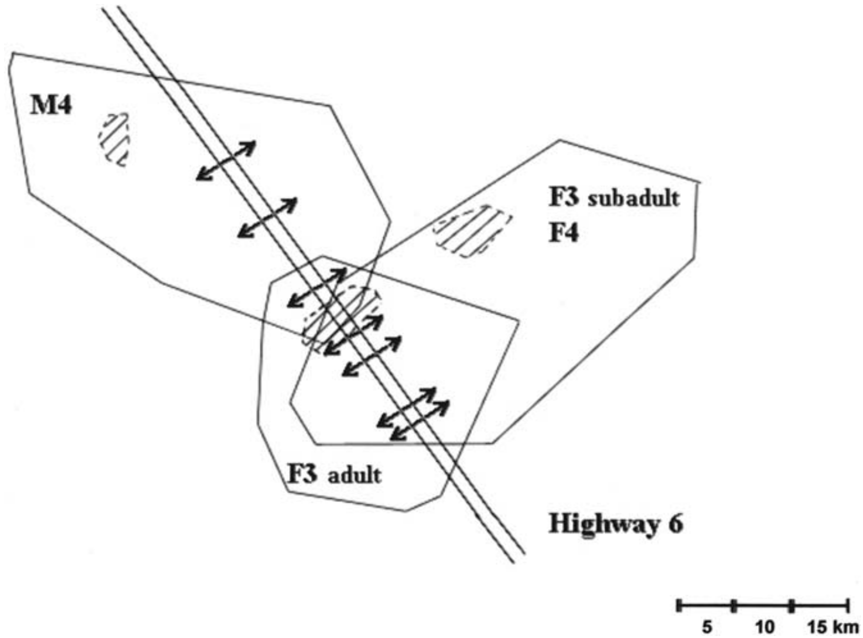
Radio-collaring and locating wolves

A lack of snow and high dog density prevented us from assessing passage use by wolves by looking for tracks. Therefore, from March 1997 to April 1999 we captured, sedated, and radio-collared 11 wolves (5 males and 6 females) in five different packs, using the procedure described in Cortés (2001). Wolves were captured at the rendezvous sites of packs previously located by simulated howling. Of these, 3 wolves (2 females and 1 male) were radio-collared <15 km from the highway and 8 were captured <5 km from the River Duero: 2 (1 male and 1 female) south of the river and 6 (3 males and 3 females) north of the river; one of the latter males dispersed soon after being radio-coallared, and settled near the highway.

We located the wolves from the ground or from aircraft using standard triangulation or homing techniques (Mech 1983). We monitored the wolves intensively near the highway from January 1998 to June 1999; F3 was also intensively monitored from August to October 2001. Wolves near the River Duero were monitored from March 1997 to February 2001. If we did not locate them in the morning at their usual refuges, we searched by light aircraft in the afternoon. We also monitored them overnight, determining a focal wolf's location by ground triangulation approximately every 15 min, starting 4 h before sunset and continuing until 2 h of inactivity had elapsed. To detect the crossing points on the highway, we waited for the focal wolves near the structures, presuming that they would cross there during overnight monitoring.

We computed home-range size by the minimum convex polygon (MCP) method (Hayne 1949; White and Garrott 1990) and the fixed kernel estimator (Kenward and Hodder 1996), using only 1 location per day per wolf to avoid

Fig. 2. Home ranges of three territorial wolves in relation to Highway 6. Hatching indicates core areas (50% fixed kernel), including mainly dens and rendezvous sites. Arrows denote locations of vehicle bridges along the highway.



pseudoreplication. The number of crossings was determined by sequential locations of individual wolves on opposite sides of the highway and the river. The percentage of crossings represents a minimum, since we were only able to detect return-trip crossings on the same day during overnight monitoring sessions.

Wolf population monitoring and assessment of habitat quality

The distribution of wolves in Spain has been studied since 1970, but the approximate number and distribution of packs were assessed for the first time during 1987 and 1988 (Blanco et al. 1992). Since then, wolf expansion in northern Spain has been regularly surveyed by different authors, who have monitored the settlement of new packs in different regions (see the review in Blanco and Cortés 2002). Wolf density in the agricultural area north of the River Duero in the late 1990s was determined by intensive radio-tracking and searching for dens and rendezvous sites by simulated howling (Harrington and Mech 1982; Fuller and Sampson 1988) over a 2000-km^2 area from 1997 to 1999 (Blanco and Cortés 2002). In 2000 and 2001 another detailed wolf survey was carried out in the Castilla y León region to locate breeding packs and determine population trends since 1988. Involving 9 biologists, the study analysed 330 mail-enquiry responses by wardens and 1258 by hunters. In addition, on 557 field working days, 2778 personal interviews with local people were conducted, 7787 km was scouted for wolf signs, and 209 sit-and-wait sessions and 879 simulated howling sessions were carried out to locate the pack rendezvous sites. Over an area of 75 200 km^2 we located 149 packs, 107 of which we consider to be definite and 42 probable (Llaneza and Blanco 2005). New pack ranges were mapped by radio-tracking when there was 1 or more radio-collared wolf in the pack (Ciucci et al. 1997). Otherwise, we mapped the area that comprised the rendezvous sites and the dens located by simulated howling, and the area where wolf signs, sightings by local people, and attacks on livestock were detected.

Habitat quality is negatively affected by road density (Thiel 1985; Mech et al. 1988; Mladenoff et al. 1995). In addition, our radio-collared wolves selected thick vegetation for diurnal resting (Cortés 2001). Accordingly, we deemed pack habitat quality to be directly proportional to forest cover and inversely proportional to road density.

Results

Impact of the highway on radio-collared wolves

Social ecology of wolves that crossed

Four radio-collared wolves were detected crossing the highway, i.e., all the radio-collared wolves living <15 km from the highway for at least 1 month. They represented all four social categories: (1) A territorial breeding female (F2), apparently >5 years old when radio-collared. From September 1997 to May 1999 her home range was 532 km^2 (100% MCP, 168 relocations), 19% west and 81% east of the highway. However, 98% of resting sites were on the eastern side of the highway, where all the dens and the rendezvous sites were located, 12–15 km from the highway (Fig. 2). (2) A young adult territorial male (M4), apparently the breeding male judging by leg-raised urination and consistent den attendance from 1998 to 2002 (Peterson et al. 2002). From May 1998 to May 1999, total home range was 490 km^2 (100% MCP, 93 relocations), 77% and 23% of his area were on the western and eastern side of the highway, respectively, but only 5% of the resting-site relocations were recorded on the eastern side. Den and rendezvous sites were 6–8 km west of the highway (Fig. 2). (3) A young adult male (M2), who was a floater from March 1998, when he was radio-collared 30 km west of the highway, to January 1999. During this period, we located him on 59 days over an area of

Table 1. Numbers of detected crossings, total numbers of days located, and crossing frequencies (detected crossings/days located) for radio-collared gray wolves (*Canis lupus*) near the highway.

Sociality	Reproductive status	ID No.[a]	Monitoring period	No. of days located	No. of detected crossings	Crossing frequency (%)
Territorial	Breeder	F2	23 Sept. 1997 – 15 May 1999	163	8	4.9
Territorial	Breeder	F3	1 Oct. 1999 – 12 Oct. 2001	72	24	33.3
Territorial	Breeder	M4	15 May 1998 – 15 May 1999	92	8	8.7
Territorial	Subadult	F3	23 Sept. 1997 – 18 Oct. 1998	91	4	4.4
Disperser	Adult	F3	19 Oct. 1998 – 15 May 1999	45	10	22.2
Disperser	Adult	M2	8 May 1998 – 15 May 1999	56	13	23.6

[a]M, male; F, female.

1632 km^2; however, his real home range could have been larger because we succeeded in locating him in only 48% of attempts. We found that 61% of the home-range area and 60% of the relocations were on the western side of the highway and 39% of the area and 40% of the relocations were on the eastern side. During his dispersal period, M2 did not use any particular area intensively. (4) Female F3 was about 16 months old when radio-collared in September 1998. She was living in F2's pack and spent most of the time with F2 before dispersing, indicating that F2 was probably her mother. During the 5 years' monitoring, F3 showed consecutively three social ranks: (1) territorial subadult living in her apparent natal pack from September 1997 to October 1998; (2) disperser from October 1998 to October 1999; and (3) territorial breeder from October 1999 to September 2001 (Fig. 2). She crossed the highway during all three stages. During the first stage, F3 shared F2's territory and was located in a home range of 488 km^2 (91 relocations), 21% of the area (2% of relocations) on the western side and 79% (97% of relocations) on the eastern side of the highway, where the pack's den and rendezvous sites were located (see Fig. 2). During dispersal, F3 was located 45 times in a 1309-km^2 area, although it could be much higher considering that we did not detect her in 59% of attempts. We found that 31% of the area and 27% of locations were on the western side of the highway and 69% of the area and 73% of locations were on the eastern side. In October 1999, F3 (3.5 years old at the time) established her territory on both sides of the highway, at least until October 2001, when intensive monitoring was abandoned. The core area occupied the less forested and most disturbed habitat of F2's home range. F3's home range area was 333 km^2 (72 relocations), 69% of the area and 71% of relocations on the western side and 31% of the area and 29% of relocations on the eastern side of the highway. In 2000 and 2001 she gave birth in May in a den 500 m west of the highway, and in August moved the pups to a cornfield 2 km north of the den and 500 m west of the highway, where the pack remained until October, when they initiated nomadic movements

The wolves that crossed the highway came from three different packs (four when F3 established her own pack), suggesting that crossing highways is not the cultural habit of any particular pack. In addition, we saw other pack members crossing the highway with the radio-collared wolves, and local people have observed other non-radio-collared wolves crossing four-lane fenced highways both in our study area and elsewhere in Spain (unpublished data). This suggests that most wolves may regularly cross highways similar to th in the study area, as well as other more permeable ones, i.e., most Spanish highways. This obviously does not mean that highways have no effect at all on wolf populations.

Crossing frequencies

Three of 4 territorial wolves with home ranges adjacent to the highway crossed it sporadically (<10% of monitoring days), apparently to explore new areas or because they were attracted by unusual food sources (e.g., rabbits with myxomatosis). The 2 dispersers crossed it on 22%–24% of monitoring days, apparently searching for an area in which to establish a territory (Table 1). From 1 October 1999 to 12 October 2001, F3 (when she was a breeding female) crossed on at least 33% of the 72 monitoring days. During 28 days of intense monitoring in August and September 2001, which included 10 periods of continuous 24-h monitoring, she crossed the highway on 39% of monitoring days. During this period, we detected her 4 times resting during the day 500–1000 m from her 5 pups, but on the other side of the highway. At night she crossed the highway back to the rendezvous site where the pups were based.

Daytime selection

From 14 crossings for which the time was known, 12 occurred at night and 2 in daylight (at 0805 and 1603). These results agree with the daily activity pattern of the radio-collared wolves, which is mainly nocturnal (unpublished data), as recorded in other studies conducted in human-affected areas (Spain, Vilà et al. 1995; Italy, Ciucci et al. 1997).

Crossing points

In 14 of 67 crossings detected, we were close enough to the wolves to determine the crossing point. In all cases they used vehicle bridges over the highway. Because all the bridges were similar, we could not identify structural characteristics that might influence selection of bridges by wolves. The most important landscape difference is the distance from bridges to villages, farms, or inhabited housing. Ten of 14 (71%) crossings were made over bridges in disturbed areas, i.e., ≤200 m from housing (Table 2). Considering that 61% of the bridges are located in undisturbed areas, the difference is significant ($\chi^2 = 6.31$, $P = 0.01$, df = 1). The most used bridge (D; see Table 2) was near a fairly active arable farm <1000 m from one packs's main rendezvous site.

Table 2. Wolf crossing points along the highway.

Bridge	No. of crossings	Crossing frequency (%)[a]	Human intrusion near bridges	Distance (km)[b]
A	1	7.1	Village and petrol station 200 m away	1.8
B	3	21.4	No dwellings within <1 km	0
C	3	21.4	1 rural house 100 m away	2.3
D	6	42.9	1 farm <50 m away	2.1
E	1	7.1	No dwellings within <1 km	0

[a]Detected crossings/days located.
[b]Distance to the nearest undisturbed potential crossing point.

Table 3. Numbers of detected crossings, total numbers of days located, and crossing frequencies (detected crossings/days located) of radio-collared wolves near the River Duero.

Sociality	Reproductive status	ID No.[a]	Monitoring period <10 km of RDA	No. of detected crossings	No. of days located	Crossing frequency (%)
Territorial	Adult	F4	10 Oct. 1997 – 28 Feb. 1998	0	34	0
Territorial	Adult	F6	1 Apr. 1999 – 29 Nov. 1999	22	51	43.1
Territorial	Subadult	M1	1 Mar. 1997 – 11 Jan. 1998	0	61	0
Territorial	Subadult	F1	23 Apr. 1997 – 23 July 1998	0	27	0
Territorial/disperser	Adult	F5	10 May 1998 – 12 July 1999; 19 Mar. 2000 – 20 June 2000	9	81	11.1
Territorial/disperser	Subadult	M5	30 Mar. 1999 – 20 Aug. 1999	7	47	14.9
Disperser	Adult	M2	8 Mar. 1998 – 8 May 1998	0	21	0
Disperser	Adult	M3	8 Mar. 1998 – 23 Oct. 1998	0	51	0

[a]M, male; F, female.

The importance of wolves' habituation to human activity

In our study area, wolves are habituated to human activity and may be less reluctant to cross highways than wolves living in wilderness areas. Three casual observations illustrate wolves' apparent habituation to highways and to human activities. On 15 March 1999 at 1602 (in daylight), M4 was observed crossing a bridge over the highway (average traffic volume 11.0 vehicles/min). After crossing the bridge, the wolf stopped¸ looked at the highway, and on seeing our stationary car on the hard shoulder, he continued walking. On 4 November 1998 at 2020, after nightfall, M2 was observed ranging and hunting (probably rabbits) in vegetation along the side of the highway, separated by just a wire fence and seemingly unaware of the traffic (traffic volume 7.4 vehicles/min). At 2210, M2 headed for the nearest bridge and crossed over the highway. In an overnight session on 10 January 1988 we followed F2 (the breeding female) and F3 (almost certainly her daughter), who were moving together, perhaps with other wolves from their pack. At 0405 they reached the highway near a village (population 462); F3 crossed the highway over the nearest bridge, located near the village and <200 m from a 24-h petrol station, while F2 took a 1.8-km detour to select another bridge in an undisturbed area. This behaviour suggests that tolerance to human activities is an idiosyncrasy of individual wolves.

Impact of the RDA on radio-collared wolves

Between 1997 and 1999, we radio-collared 8 wolves in two packs living <5 km from the River Duero. The packs were 100 km apart on different sides of the river (north and south); the core area of the north pack was a 15-km^2 forest belonging to the Spanish Army. Six of these 8 wolves dispersed during the study period; on average, the radio-collared wolves stayed <10 km from the river for 7.1 months (range 2–17 months). Five of them were never detected crossing the river before dispersing or dying (Table 3). One of them (F4) was poisoned and died in the same area where she was radio-collared, and the remaining 4 dispersed; one of the latter (M3) was killed by a car during dispersal and the 3 other wolves (M1, M2, and F1) successfully established a territory farther from the river but on the same side where they were radio-collared.

We detected only 2 river crossings prior to March 1999 and 32 crossings from April to July 1999. Between March 1997 and March 1999 (252 monitoring days) we detected only 2 crossings. In January 1999, dispersing female F5 made one round trip in an area where the river is 30 m wide and shallow with no infrastructures or disturbed areas that could cause an additional barrier effect. In March and April 1999, we radio-collared yearling male M5 and barren adult female F6 in the Army-owned forest north of the river; also, F5 returned to this forest after an 8-month dispersal period. On 28 April 1999 we detected F6 for the first time south the river, and the same night she crossed again north to the Army-owned forest. On 8 May 1999, exceptionally extensive military maneuvers took place, involving hundreds of soldiers and real artillery fire. When they finished 1 week later and we were allowed to resume the research, the 3 radio-collared wolves that regularly inhabited the forest (F5, M5, and F6) were south of the River Duero. During the next 3 months the 3 radio-collared wolves crossed the river regularly, sometimes twice (round trip) on the same night. In this period, F5 crossed on 19% of the 16 monitoring days, and M5 and F6 crossed on 25% (n = 28) and 71% (n = 31) of the days, respectively. In total, the 3 wolves crossed on 43% of the 75 monitoring days. In August 1999, F5 and M5 dispersed south of the River Duero, where F5 was illegally shot in February 2001 and M5 disappeared at the end of August 1999; F6 spent most of the time north of the river, where she was illegally killed in January 2000.

Crossing the RDA is a complex matter, since wolves must negotiate all the obstacles described above (see the Barriers section in Methods). We suggest that F6 had learned to find the way through these obstacles prior to the military maneuvers. When the other 2 radio-collared wolves were seriously disturbed by the maneuvers, they followed F6 (or another non-radio-collared wolf who knew how to cross the river artery) and learned how to cross. After that, they crossed regularly and eventually dispersed south of the River Duero, where wolf density was much lower.

Wolves crossed 7 times during 24-h monitoring sessions, always at night. We were unable accurately to locate the crossing points, but we suspect that they used vehicle bridges, although one wolf apparently once crossed a small dam containing only shallow water.

These results indicate the important role of learning, and suggest that an accumulation of obstacles may have synergistic effects, causing a barrier effect greater than the sum of the effects of the individual ones.

The apparent influence of highways and the RDA on population expansion

In 1970 the wolf population in northwestern Spain started to expand southwards and eastwards, occupying new areas (Blanco et al. 1992; Blanco and Cortés 2002). This has provided the opportunity to assess the relative influence of highways and the RDA on population expansion.

The wolf population has been regularly monitored since 1988 and there is no evidence that highways have prevented or retarded wolf expansion. Since 1988, the expanding population has reached 5 four-lane fenced highways and breeding packs have established themselves almost simultaneously on both sides of the highways (Fig. 3), so the highways do not have any apparent influence on population expansion. Only the Basque Region highway has not been crossed by the breeding population since 1988, when the first breeding pack was detected west of it, this being due to severe culling to rid the region's free-ranging sheep areas of wolves (Sáenz de Buruaga et al. 2000).

In contrast, analysis of expansion of the wolf population as a whole strongly suggests that the RDA has delayed that expansion for about 15 years. When the Spanish wolf population started to expand, it reached the River Duero, in the west of its range, at the end of the 1970s, and the central and eastern parts in the 1980s. During this period at least 7 lone male wolves were sporadically killed 50–100 km south the river (Blanco et al. 1992), but no breeding packs were detected. Those individuals were probably dispersers exploring distant areas before returning to the breeding range, an apparently usual behaviour described by Merrill and Mech (2000). After becoming established, in the late 1980s and the 1990s the population immediately north of the River Duero showed obvious signs of increasing. In 1988, density was estimated at 0.4–0.6 wolves/100 km^2 (Blanco et al. 1990), and in 1999 at 2.5–3.0 wolves/100 km^2 (Blanco and Cortés 2002). By the late 1990s, this population seemed to be saturated, as suggested by the following: (*i*) our radio-collared wolves spent 41% of the study period as solitary floaters, i.e., dispersers unable to find a new territory (in saturated American populations, solitary wolves make up 29% of the population; Fuller 1989); (*ii*) the newly established packs that we detected (including those formed by radio-collared wolves) settled in areas of very poor quality, which suggests that the most suitable habitats were already occupied.

During this period, the population immediately south of the River Duero grew very slowly. In 1988 and 1990 the first two breeding packs were detected almost 200 km apart, and until 1997 we knew of no new pack. The next new packs were detected from 1998 onwards, and from 1997 to 2001 the area occupied by the population suddenly expanded by about 20 000 km^2. Finally, in the survey carried out in 2001 in Castilla y León, 20 packs were detected and we estimated a density of 0.5 wolves/100 km^2 south of the River Duero (Llaneza and Blanco 2005). This sudden increase after a long period of stagnation is similar to the expansion of partially isolated wolf populations studied in Montana (Boyd et al. 1995), Wisconsin (Wydeven et al. 1995) and Scandinavia (Wabakken et al. 2001). In the first two cases, dispersers from the source populations were impeded by disturbance to a large part of the habitat. The Scandinavian wolf population is partially isolated from the Finnish source population by the semi-wild reindeer management zone, a large area in Norway, Sweden, and Finland where wolf packs are legally removed.

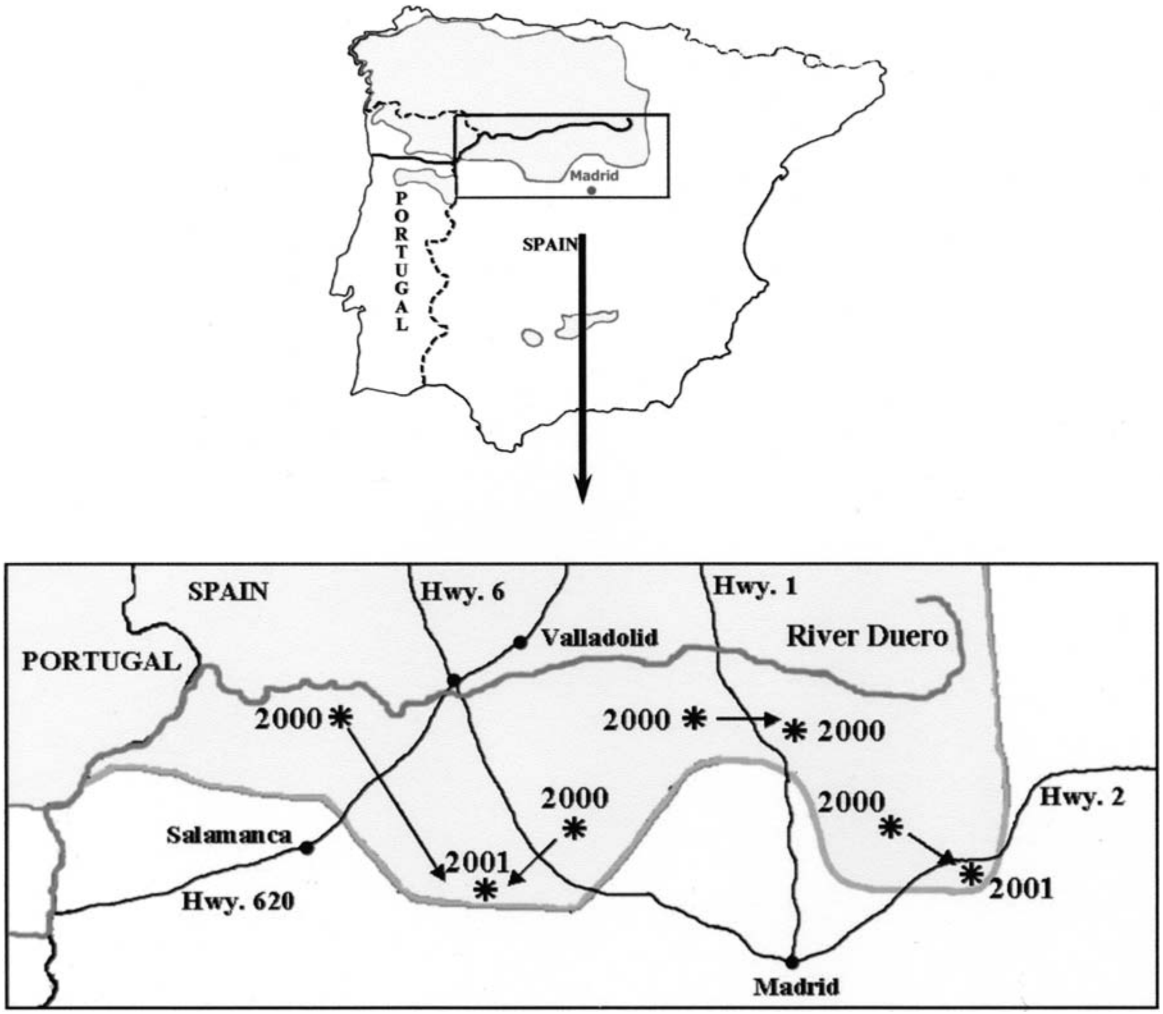
It is unlikely that the delay in wolf expansion south of the River Duero was due to poor habitat quality or high wolf mortality. Habitat is very similar on both sides of the river in all aspects except refuge availability: forest cover is much higher south (26%) than north (7%) of the River Duero (Cortés 2001). In addition, south of the River Duero wolves are fully protected by the European Union's Habitat Directive, but are classed as a game species north of the river. So the most likely reason for the delay in wolf expansion is the barrier effect of the RDA.

Discussion

This study, conducted in a flat, treeless agricultural environment, showed that wolves regularly crossed a fenced four-lane highway with no wildlife-crossing structures and an average traffic volume of 12 312 vehicles/day, and yielded no evidence that either the study highway or similar Spanish highways limited or delayed wolf population expansion. In contrast, 5 of 8 radio-collared wolves were not recorded as crossing the RDA, and 2 of the remaining 3 wolves began crossing regularly only after being forced to do so by serious disturbance, which suggests that it may act as an important barrier at the individual level. Analysis of the expansion of the wolf population as a whole since 1970 strongly supports this conclusion, since there is sound evidence that the RDA has been delaying wolf population expansion in Spain for almost two decades.

In the area of the two study packs, the River Duero itself does not seem to present a much greater barrier effect than a four-lane fenced highway. It is not much wider and can be

Fig. 3. Influence of Highways 1, 2, 6, and 620 on wolf expansion. The asterisks indicate packs detected in highway environs and the year when they were first detected.



crossed via bridges or dams or even by swimming. The actual difference is that the river is flanked along most of its course by other small lineal barriers such as two-lane unfenced roads, railways, channels, strips of disturbed habitat, etc., which alone would not pose any problem to wolves crossing, but acting synergistically, appear to multiply the barrier effect on the population.

Previous studies carried out in the United States have indicated wolves' ability to cross highways, although the latter were more permeable than the highway in our study area. Wolves in Banff National Park regularly cross the four-lane fenced highway that bisects the park and has an average summer traffic volume of 20 000 vehicles/day (Gibeau and Herrero 1998). However, they use wide underpasses built for wildlife every 2 km, on average (Clevenger and Waltho 2000). According to Clevenger (1999), wolves crossed the highway through underpasses 82% of the time that they approached them, a higher rate than that previously quoted by Paquet and Callaghan (1996) (between 44% and 83%), who monitored 2 underpasses in the same park. In Wisconsin, Kohn et al. (1999) concluded that the enlarged US Highway 53, a four-lane highway with a traffic volume of 4700 vehicles/day, did not act as a barrier to dispersing wolves. As it was not fenced, it was more permeable than the one in our study area. In addition, in both Banff National Park and Wisconsin, the area surrounding the highway is almost undisturbed, at least compared with our densely populated study area in Spain.

To cross fenced highways, wolves use underpasses in Banff National Park (Clevenger and Waltho 2000) and overpasses in Spain, as they are the most available structures in each area. In Banff National Park, carnivores select the underpasses farthest from towns and with least human activity (Clevenger and Waltho 2000). This seems to be a rule for carnivores, since foxes, *Vulpes vulpes* (L., 1758), and wild cats, *Felis silvestris* Schreber, 1775, consistently avoided crossing a high-speed railway through passages located near permanent sources of human disturbance (Rodríguez et al. 1997), and dispersing cougars in California avoided areas with artificial light at night (Beier 1995). In contrast, our radio-collared wolves did not avoid human presence so obviously when selecting crossing points on the highway, using bridges located less than 200 m from inhabited houses with outdoor lighting in a higher proportion to their availability. As there is one bridge every 2 km (Table 2) and wolves walk at an average speed of 8.7 km/h (Mech 1994), a 4-km detour would take 27 min. But wolves apparently selected the bridges closest to dens and rendezvous sites, and preferred to walk near inhabited houses rather than make a short detour to seek more undisturbed bridges.

The presence of food sources (i.e., carrion pits, garbage dumps) seems to reduce wolf avoidance of human activity (Paquet and Callaghan 1996). In our study area, livestock carcasses averaged 75% of the food biomass taken by wolves (Cortés 2001), and human activity is widespread throughout the wolves' home ranges, including the areas around dens and rendezvous sites. Wolves habituated to human activity may be less reluctant to cross highways than wolves living in wilderness areas. The cases described above, including 1 radio-collared wolf crossing the highway in daylight apparently undisturbed, another hunting for hours along the roadside, and other casual observations, seem to support this opinion. Wolf adaptation to disturbed habitats is crucial to an understanding of wolf recovery in America and Europe over the last few decades (Mech 1995). Wolves can gradually get used to highways and other disturbed habitats in a process that may take a few generations and is illustrated by the change in F3's behaviour. When she matured, she established her home range on both sides of the highway, given three circumstances. Fist, she was familiar with the highway and the overpasses, since she had visited them sporadically in previous years with her native pack; second, the area was apparently saturated with wolves, with new pairs being obliged to settle in very disturbed habitats; and third, F3 seemed to be an especially fearless wolf, as suggested by her aggressive behaviour when captured, the fact that we observed her attacking a sheep flock in daylight with the shepherd present, and her use of much more disturbed habitats than the other radio-collared member of her pack.

The idiosyncrasy of individual wolves may influence adaptation to disturbed habitats by themselves and their offspring. This individual variation has been stressed in virtually all studies on wolf behaviour in captivity (Rabb et al. 1967; Fox 1971; Zimen 1981) and in some studies in the wild (Peterson et al. 2002). Paquet and Callaghan (1996) reported that the death of a breeding female drastically reduced the use of an underpass by a wolf pack in Banff National Park, and stated, "habituation and social transmission of information may be important in establishing consistent usage of underpasses". After settling her territory on both sides of the highway, F3 reared 6 pups in 2000 and 5 pups in 2001, who were living less than 1000 m away from the highway at least from May to October, and were therefore particularly habituated to living around highways. The genetic and cultural features of such wolves are likely to prevail in environments with highways.

Wolves are adaptable, social animals and fast breeders (Mech 1995), so they have more opportunities to learn from other individuals in the same group and to transmit information to a larger number of offspring. Among large carnivores, brown bears are in the opposite situation in that they are solitary and have a lower reproductive rate. They seem to have more difficulties in adapting to highways. In a sample of more than 5000 radio relocations of 51 bears from 1996 to 2001, Gibeau and Herrero (1998) found that no radio-collared females crossed the Trans-Canada Highway (20 000 vehicles/day in summer) in Banff National Park; 5 male bears crossed, but only 1 did so regularly. In the same area, Clevenger (1999) reported that over a 35-month monitoring period, the crossing structures was used for cross-highway travel 355 times by black bears, 351 times by cougars, 256 times by wolves, and over 3000 times by coyotes, but only 15 grizzly bear passes were detected, 9 of them by 3 known radio-marked adult males and 6 by unknown individuals. In Slovenia, Kaczensky et al. (2003) found that only 3 of 15 radio-collared brown bears living within 10 km of the four-lane fenced Ljubljana–Razdrto highway (7500 vehicles/day) crossed it 7 times, and all were subadult bears.

The fact that wolves are able to cross the highways in our study area regularly, however, does not imply that highways do not pose any problem to wolves. Fenced highways probably redirect wolf movements to crossing points, and some individuals refuse to use these facilities (Paquet and Callaghan 1996; Clevenger 1999). In addition, wolves are hit by vehicles, even on fenced highways. In our study area not one radio-collared wolf has been killed by a vehicle on the highway, but 3 non-collared wolves were killed by cars in the 58-km section that we monitored from 1997 to 2000, and 3 of the 11 radio-collared wolves were killed by cars on secondary unfenced roads or tracks. In Banff National Park, 16 wolves were killed by cars during a 18-year period, 81% and 19% along unfenced and fenced sections, respectively (Clevenger et al. 2001*b*). On unfenced US Highway 53 in Wisconsin, 1 of 59 radio-collared and 2 non-radio-collared wolves were killed by vehicles from 1994 to 1999 (Kohn et al. 1999). However, these studies indicate that the disturbance and mortality caused by highways do not seem to pose a serious threat to wolf populations.

In contrast, the barrier effect caused by the RDA is much more important than that of the study highway, since in Spain it has delayed the expansion of the wolf population for almost two decades, and in Portugal — where the habitat is more disturbed and the river is wider, as it is closer to its mouth — the artery seems to isolate the small and scattered southern population (consisting of about eight packs) from the dense northern population (Moreira 1998). In Spain in the 1980s and most of the 1990s, several lone wolves and two packs almost 200 km apart were detected south of the River Duero, but the population did not increase for more than 15 years (Blanco and Cortés 2002).

We can think of only 3 plausible explanations for this period of wolf population stasis south of the River Duero, followed by rapid expansion: (1) poor quality and distribution of habitat, (2) differences in persecution level, and (3) reduced immigration and, owing to the barrier posed by the RDA, an Allee effect, which predicts a decrease in reproduction and (or) survival when conspecific individuals are not numerous enough (Courchamp et al. 1999; Stephens and Sutherland 1999), mainly in social species (Courchamp et al. 2000). Habitat quality does not seem to be poorer south of the river. Forests account for 7% of habitat north of the River Duero, but 26% south of the river, and represented 42% of the home ranges of radio-collared wolves living south of the river (Cortés 2001; J.C. Blanco and Y. Cortés, unpublished data). We have not detected any differences in other habitat characteristics, including food availability, on the two sides of the river. Moreover, wolves are fully protected south of the River Duero, whereas there is a small hunting quota in the north. Nor does illegal persecution seem to be higher in the south. Among our radio-collared wolves, only 1 of 4 (25%) was illegally killed in the south, compared with 3 of 7 (43%) in the north.

The stagnation of the population for almost two decades could be due to the Allee effect.Thus, the trend of the population south of the River Duero — less than 1 km from the northern population — was almost identical with that shown by the recently established wolf population of Scandinavia, which was formed from a few dispersers from Finland, 1000 km away (Wabakken et al. 2001; Vilà et al. 2002). The Scandinavian population stagnated because of the effects of inbreeding and (or) incest avoidance, but the arrival of a single new male from the Finland source population triggered a rapid flourishing of the population (Vilà et al. 2002). In our study, the regular arrival of wolves south of the River Duero suggests that the 15-year stagnation may have been due to conspecific attraction (Reed and Dobson 1993), which would have discouraged the dispersers from settling south of the river, where there were hardly any conspecifics. In the absence of the barrier effect of the RDA, population expansion would probably have been as regular as it was north of the river.

This study suggests that the addition of several small barriers can have synergistic effects, creating a much stronger barrier effect. So far, highways have not exerted an obvious effect on wolf populations in Spain, but many of them are of recent construction and are progressively adding petrol stations, hotels, and other facilities, which can increase the barrier effect. Furthermore, the number of four-lane fenced highways is increasing rapidly. In a 25 000-km^2 area that includes our study area, there was 175 km of highway in 1997, and 635 km more is planned by 2007. These barriers, acting together, may have had an impact as yet undetected in single highways. We therefore recommend building wildlife crossings as a routine measure in any new infrastructure, following the procedures described in the scientific literature reviewed in this paper. The small Portuguese wolf population south of the River Duero has been decreasing or stable over the last 30 years, in contrast to the flourishing population north of the river (Grilo et al. 2002). Since the RDA is probably an important barrier, natural contact with the emerging Spanish population south of the river should be promoted to allow this Portuguese population to recover. The River Duero is the only major river within the gray wolf's range on the Iberian Peninsula. However, a pattern of obstacles similar to that described in this paper is common along a large part of other major Iberian and European rivers, which may be important barriers for wolves and other large mammals. There are so many different combinations of potential obstacles that it is difficult to propose standard modifications to minimize the barrier effect of the river arteries. However, we suggest identifying the least disturbed areas along the major rivers and keeping them as permeable as possible as part of the corridors for large mammals that should be actively preserved in fragmented landscapes.

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